

Research and Technical Notes

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## RESEARCH NOTES

Auerbach, C. Mutation tests with pyronine B.

Clark's (1953) finding that pyronine B, when mixed with the food of larvae, has a weak but definitely positive mutagenic action has been confirmed. In contrast to formaldehyde food, which acts selectively on early primary spermatocytes and produces few or no mutations in spermatogonia, pyronine food acts also--or perhaps preferentially--on spermatogonia. This is shown by two observations: (a) the similarity of mutation rates in successive broods from males treated throughout larval life, and (b) the occurrence of bunches of identical mutations in the same treated testis.

Auerbach, C. The brood pattern of X-ray-induced rearrangements.

Spermatids have been shown to be more sensitive to the mutagenic action of X-rays than mature sperm. If this increased sensitivity applied equally to all genetical effects, the result of treating spermatids with a given dose of X-rays would be equivalent to that of treating spermatozoa with a higher dose. In particular, the ratio of large rearrangements to lethals in spermatids should be higher than in spermatozoa by a factor which can be calculated from the 3/2 power dose-effect law for rearrangements. An even larger increase of this ratio would be expected on Lüning's assumption that the excess of mutations produced in the sensitive cells is entirely due to intergenic changes. In an attempt to decide between these two possibilities, a number of genetical effects were scored simultaneously in successive broods from males treated with 2500 r. As further work along this line will have to be abandoned for the present, a summary of the main results obtained so far is presented here. The data are not sufficient for a decision on the point of issue; they only show that a marked increase in frequency occurs simultaneously for all studied effects.

Expt. I. (Treated ♂ Ore-K, ♀♀ y v f; dp; e. Mass matings. Mating periods of 4 days. Scored: translocations X, 2, 3; large deletions in ♀♀; small deficiencies--M; dp; e--in ♂♂.)

Brood	Translocations		Large deletions		Small deficiencies	
	n	%	n	%	n	%
1	808	4.8	appr. 4000	0.5	appr. 4000	0.8
2	707	9.5	appr. 2000	1.3	appr. 2000	1.8

Expt. II. (Treated ♂ Ore-K, ♀♀ Muller-5; dp; e. Mass matings. Mating periods of 4 days. Scored: translocations 2-3; sex-linked lethals.)

Brood	Sex-linked lethals		Translocations		Ratio L:T
	n	%	n	%	
1	610	4.8	350	2.3	2.1
2	305	10.0	144	10.4	1.0
3	707	3.3	671	0.4	8.1
4	543	0.9	347	0	

In Expt. II translocation frequency increased from the first to the second brood approximately as the square of lethal frequency. This is more than expected on the 3/2 power dose-effect rule, but the excess is not significant. The disproportionately steep decline in translocation frequency

from the second to the third brood indicates utilization in the third brood of spermatozoa which had been irradiated before meiosis, followed by loss of translocations through meiotic segregation.

Baker, William K. A chromatid translocation induced in mature sperm.

Irradiation of  $y, X^{cl}/sc^8.Y$  males with 3000 r of X-rays and mating to  $y v; bw$  females produced a fertile male having vermilion eyes but bilaterally mosaic for

yellow. (Out of 99  $F_1$  yellow vermilion males produced in this experiment, only one, aside from this mosaic, has proved fertile.) The non-yellow side of the mosaic had slightly hairy wings and small bristles. When crossed to  $y v; bw$  females the mosaic male produced 54  $v; bw$  males, 56  $y v; bw$  females, 1  $v; bw$  female, 19  $v$  males, and 29  $v$  females. All flies in the last two classes had small bristles and the slight hairy-wing effect. These data indicate that a  $Y;2$  translocation has taken place. Tests of the  $F_1 v$  males indicated that they contain the  $y^+$  marker not only on the  $sc^8.Y$  chromosome but also on the  $2^Y$  translocated chromosome. The abnormal bristles and wings of these males are undoubtedly caused by the hypoploidy of the tip of  $2R$ . The  $F_1 v; bw$  males were shown to have only the normal  $sc^8.Y$  chromosome. The only plausible explanation of the event leading to this mosaic is that a chromatid exchange had taken place, with the break in the  $sc^8.Y$  being proximal to  $y^+$  and the break in chromosome 2 distal to  $bw$ . The segregation in the first cleavage division was such that the normal 2 and the  $Y^2$  chromatids went to one nucleus, producing the sterile yellow vermilion side of the mosaic, while the  $2^Y$  and  $sc^8.Y$  chromatids went to the other nucleus, producing the fertile non-yellow vermilion half of the male. Most likely this exchange took place in mature sperm, since the exposed male was mated immediately after irradiation for only 24 hours to a single female and to a second single female for another 24 hours. The mosaic male was in the progeny of the latter mating.

Baldazzi, P. Variability of quantitative characters in strains of D. subobscura.

It is well known that strains of a *Drosophila* species collected in different localities exhibit morphological differences that are due to polygenic variability. Several strains of D. subobscura, collected in different European localities and kept for 5-6 years under uniform laboratory conditions, were examined to determine whether such variability was still maintained. With respect to Sturtevant's index of wing dimensions, a statistically significant variability among strains and sexes was found. On the other hand, no significant difference between the right and left wing of individuals was observed. No relation was detected between the variability of the Sturtevant indexes studied and different geographical and climatic factors, such as latitude, altitude, temperature, density of air, or hours of sunshine.

Barigozzi, C., and Di Pasquale, A. Further investigations on pseudotumors in *Drosophila*.

been studied. The incidences are shown below, together with the symbols used to designate these stocks:

A2-- $\delta\delta$  61%;  $\text{QQ}$  67%  
B3-- $\delta\delta$  87%;  $\text{QQ}$  81%  
C4-- $\delta\delta$  90%;  $\text{QQ}$  100%  
D -- $\delta\delta$  54%;  $\text{QQ}$  45%

The investigations begun during the last year on pseudotumors in D. melanogaster have been developed. Four new spontaneous stocks having a very high incidence have

Large variations occur, which can greatly change the difference between sexes. Crosses between the four stocks gave in general a low production of pseudotumors. The highest incidence was obtained from the cross A2 x D, the lowest incidence from A2 x B3. This proves that the genotypes of A2 and D are more similar than those of A2 and B3. C4 gave a higher incidence, because it behaved as a dominant, in all crosses made.

All four stocks have been studied, using balanced lethals, to test the activity of the first, second, and third chromosomes in producing pseudotumors. In A2, B3, and D, the only genotypes which are able to produce pseudotumors are those carrying the second chromosome of the tu stock. Chromosomes 1 and 3 seem to act only to modify the incidence. Stock C4 behaves differently, because the first chromosome is also involved (to nearly the same degree as the second), and the third has very little effect. Thus in A2, B3, and D the genes for pseudotumors are relatively recessive and are located only in the second chromosome. In C4 the genes are semidominant and are located mainly in the first and the second chromosomes.

Selection for high and low incidence of tumors in stocks having only the second tu chromosome seems to give positive results, thus proving that this chromosome contains many genes, which have a cumulative effect.

To test whether the cytoplasm to some extent influences the action of these genes, two different experiments were devised: first, the transfer of tu chromosomes into tumorless egg cytoplasm, by means of appropriate crosses; second, the transfer of chromosomes from a tumorless stock into egg cytoplasm from a tumorous stock. The first combination gave pseudotumors immediately, showing that a particular kind of cytoplasm is not needed for this expression of chromosomal activity. The second combination also gave small frequencies of pseudotumors (1-4%); further data are needed for a full account of the phenomenon, but the hypothesis that the cytoplasm is also involved is justified.

Stocks of D. simulans carrying pseudotumors are also under investigation. Interspecific crosses (melanogaster x simulans) give pseudotumors.

Barish, N., and Fox, A.S.  
Antigenic effects of the  
vermilion pseudoalleles  
in D. melanogaster

An antigenic analysis of a series of coisogenic stocks differing with respect to the vermilion segment of the X has served to identify three specific components distributed as follows.

Genotype	Antigenic Component		
	V-1	V-2	V-3
Wild	-	-	haptenic
v <sup>1</sup> /v <sup>1</sup>	+	-	-
v <sup>36f</sup> /v <sup>36f</sup>	+	+	-
v <sup>48a</sup> /v <sup>48a</sup>	+	+	+
v <sup>1</sup> v <sup>36f</sup> /v <sup>1</sup> v <sup>36f</sup>	+	+	+
v <sup>1</sup> /v <sup>36f</sup>	+	+	-
v <sup>1</sup> v <sup>36f</sup> /+	+	+	-

Genetic tests (see Linkage Data, Report of N. Barish) demonstrate that the X-ray-induced mutant v<sup>48a</sup> (Fox, DIS-22) exhibits crossing over with v<sup>36f</sup> but not with v<sup>1</sup>, and must therefore be considered an allele of v<sup>1</sup> (although the possibility that it occupies a third locus is not excluded).

The results support the following conclusions: (1) The partial similarity

of the antigenic effects of these mutants are consistent with the view that pseudoalleles arise by a process of longitudinal genic replication accompanied or followed by physiological differentiation. (2) The complexity of the antigenic distribution among the genotypes studied makes it unlikely that these pseudoallelic loci are concerned with successive steps in a chain of reaction. (3) The identity of the antigenic effects of *cis* and *trans* heterozygotes, as in the case of the lozenges (Chovnick and Fox, 1953, PNAS 39: 1035), indicates that position effect is not a necessary physiological property of pseudoalleles at all phenotypic levels.

In addition to these conclusions, the following observations are of interest (1) Whereas antigen V-3 is present in two mutant genotypes in complete form (probably protein), it is present in wild in haptenic form (probably nonprotein). This may be an actual case of one mechanism of gene action proposed by Sewall Wright some years ago. (2) The fact that V-3 is produced by the single mutant  $v^{48a}$  and the double mutant  $v^1 v^{36f}$ , but not by  $v^1$  or  $v^{36f}$  alone, may provide some insight into the complexity of mutational pattern of pseudoallelic and conventional loci.

Rasden, E. B. An attempt at permanent sterilization of *D. subobscura* by X-rays.

Adults of both sexes of *D. subobscura*, stock 375 (vermillion, short vein II) aged 0-1, 1-2, 2-6, and 6-8 days were irradiated with doses of 8,000 r, 10,000 r, and 15,000 r units, generated at constant 70 kV, 7 mA, at an intensity of 90 r per 32 seconds, which was reckoned to be 1,000 r per 360 seconds. The 0-1-day-old flies were not given the 8,000 r, and so the four age groups with three doses gave eleven distinct treatments, with at least 50 adults each. Each lot was divided equally between 3 culture bottles: (a) irradiated control, (b) irradiated flies plus nonirradiated males from laboratory stocks, (c) ditto, females. This gave thirty-three distinct treatments. Controls of nonirradiated stock flies were also run. The adults were transferred to fresh bottles at weekly intervals over a 6-week period.

Briefly, the results showed that doses of 8,000 r and 10,000 r did not sterilize any of the age groups, though decidedly fewer  $F_1$  progeny emerged from 0-1- and 1-2-day-old irradiated controls (a), and from 0-1- and 2-6-day-old with males (b), at 10,000 r (29, 49, 60, and 67 flies, respectively).

At 15,000 r none of the irradiated controls (a) gave progeny except the 1-2-day age group (1 ♂, 4 ♀). At 15,000 r the addition of nonirradiated males (b) gave nil  $F_1$  with 0-1-day age group; 5 (2 ♂, 2 ♀, 1 not sexed) with 1-2-day; 1 ♂, 2 ♀ with 2-6-day; and 2 ♂, 1 ♀ with 6-8-day. At this dose the addition of virgin nonirradiated females (c) gave, in the different age groups, 49 (0-1-day), 26 (1-2-day), 46 (2-6-day), and 7 (6-8-day)  $F_1$ , as compared with an average of 298  $F_1$  in each treatment at 8,000 r and of 228  $F_1$  at 10,000 r (excluding those at 10,000 r already mentioned) over the same period.

Although the nonirradiated stock controls gave an average of 766  $F_1$  over this period, they were used primarily to prove productivity and not for a comparison of progeny numbers. Even so there was evidently some depression of population at 8,000 r and 10,000 r.

$F_2$  progeny were obtained from all 8,000-r and 10,000-r treatments, and from all the 15,000-r to which nonirradiated females (c) were originally added. Of the other 15,000-r treatments that gave  $F_1$ , only the 1-2-day age

group with nonirradiated males (b) produced  $F_2$  adults. The others no doubt failed because the numbers of  $F_1$  were so small and mold in cultures was then prevalent.

To summarize, a dose of up to 15,000 r is not sufficient to prevent D. subobscura from producing viable offspring when in contact with nonirradiated flies, for example, wild populations.

Basden, E. B. Wild aberrations in British Drosophilidae.

grouped below, the names used being purely descriptive as none has been tested genetically. Some may be due to developmental accidents or are phenocopies; but those characters marked with an asterisk are inherited in my cultures. All examples were caught wild or are  $F_1$  of wild parents that oviposited in nature. Identifications are according to Basden, 1954 (Tr. Roy. Soc. Edinb. 62: 603-654). L = left side; R = right side.

Wings

1. R wing shorter than L.
2. L wing truncated at tip.
3. Wings outspread, drooping; some L veins short.
4. \*Veins II, IV not reaching costa, and no or short post. X vein. R & L.
5. \*Vein II not reaching costa. R & L.
6. L vein IV not reaching costa.
7. Vein V short, post. X vein near ant. X vein. R & L.
8. Vein II branched at tip.
9. Vein IV with extra pegs, post X vein abnormal. R & L.
10. \*Post. X vein incomplete.
11. Post. X vein distorted or branched.

Bristles

12. No apical scutellars.
13. One apical, three lateral scutellars.
14. Extra scutellar.
15. Extra humeral.
16. Strong mid-sternopleural.
17. Two strong palp bristles.
18. Extra frontal bristle.
19. No body bristles.

Although no special search was made for them, some morphological aberrations and color variations were noticed in wild British Drosophilidae. A selection is

grouped below, the names used being purely descriptive as none has been tested genetically. Some may be due to developmental accidents or are phenocopies; but those characters marked with an asterisk are inherited in my cultures. All examples were caught wild or are  $F_1$  of wild parents that oviposited in nature. Identifications are according to Basden, 1954 (Tr. Roy. Soc. Edinb. 62: 603-654). L = left side; R = right side.

Obscura, ♂. Subobscura, ♂. Reared only.

Subobscura, ♀.

Obscura, ♀ (see also Nos. 27, 32). Reared only.

Ambigua, ♂♀. Reared only.

Subobscura, ♂♀.

Subobscura, ♀. Reared only.

Subobscura, ♀.

Obscura, ♀, R (reared). Immigrans, ♀, L.

Subobscura, ♂. Reared only.

\*Subobscura, ♂, L; ♀, R. Silvestris, ♂, R. Obscura, ♀, R. Reared only.

Phalerata, ♀, R & L. Ambigua, ♂, R.

Funebris, ♂, R.

Scaptomyza apicalis, ♂. Reared only.

Deflexa, ♂.

Melanogaster, ♀ (5 scutellars).

Vibrissina, ♂ (3 strong humerals, L).

Subobscura, ♂♀. Obscura, ♂♀. Normally only the ant. and post. sternopleurals are evident.

Obscura, ♂♀. Normally only 1, at tip. Two strong bristles are characteristic of D. tristis.

Scap. graminum, ♀, R & L. The extra frontal bristle is characteristic of S. ?montana.

Obscura, ♂ (reared). Hydei, ♂ (Kent).

Ovipositor plates (guides)

20. Ovip. plates black. Forcipata. Normally yellow.  
 21. Dorsal long bristle missing. Silvestris. Reared only.  
 22. Ventral long bristle duplicated. Silvestris; Obscura.  
 23. Ovip. plates deformed, Subobscura. Reared only.  
 lightly chitinized.

Legs

24. All tarsi 4-jointed; only 1 sex comb on each front tarsus.  
 25. R hind 3rd tarsal joint dilated.  
 26. Hind tibia curved; hind tarsi swollen.  
 27. Legs generally deformed.

Color

28. No yellow on abdominal tergites.  
 29. \*Vermilion eyes.  
 30. Trident mark.  
 31. Eyes, small.  
 32. Arista, duplicated.  
 33. Abdomen twisted, L half of 3rd tergite much narrowed with no black marking; mark reduced on L of 4th tergite.  
 34. Three spermathecae.

Obscura, ♂.Subobscura, ♀.Subobscura, ♀. This is similar to D. ingrata Hal. (1833) which is probably an aberrant D. subobscura (Basden, DIS-27: 81).Obscura, ♀ (= No. 3). Reared only.Obscura, ♀. (D. obscura and D. bifasciata females are usually distinguished by the presence or absence, respectively, of abdominal yellow. Frequent cases with no yellow have been checked by breeding and all have been D. obscura.)Subobscura, ♂ (reared). Funebris, ♀.Melanogaster, ♂.Melanogaster, ♀ (Benenden, Kent).Obscura, ♀, L (= No. 3). Reared only.Phalerata, ♀.Subobscura. It has been noticed that a short vein III has not been found and that only the female of D. phalerata showed aberrant posterior X vein, in wild specimens.Bateman, A. J. Attached-X  
Muller-5.

The second was mated first to y Hw mg d-49 males and then, on proving very infertile, to wild-type males. Eventually a few eggs were laid. These yielded only 5 flies: 1 y Hw mg d-49 male and wild-type males. Evidently the female was attached-X as a result of failure of division of the centromere at the second division of spermatogenesis in a Muller-5 male. For some reason this conformation was both highly sterile and of low viability.

Bateman, A. J. Deleted X's as a measure of the mutagenic sensitivity of developing sperm.

bearing a deleted X) varies greatly with the day of mating. After 1000 r the frequency of hyperploids from mature sperm (1st day's mating) is less than 0.1%. But the frequency rises daily, until it is over 4% (40-fold increase)

In the course of sexing thousands of flies from the cross y x M-5, two M-5 females have been found on separate occasions.

If one-day-old wild-type males are irradiated and mated to fresh virgin y females daily, the rate of production of hyperploid non-yellow daughters (the result of sperm

on the 7th-to-8th days, an increase which corresponds to meiosis. It then drops to even less than the initial value, though X-Y and X-autosome translocations in premeiotic stages could also produce hyperploidy of similar phenotypes.

Bateman, A. J. Mutations produced by phosphorus-32.

The P32 content of the flies was measured at various stages. The rate of hyperploid production was proportional to the P32 content of the fly 6-7 days prior to mating, for males that pupated outside of the active medium. Following pupation in active medium, there was an additional burst of mutation in sperm maturing 7 days later. This increase was about equal to that due to internal P32 of the pupa. Thus ingested and external P32 were of about equal mutagenic importance to the pupa.

Bateman, A. J. The effect of storage of sperm in the male on mutation rate.

Lüning (1952), using the dominant-lethal method, claimed that if males are kept unmated for a period after irradiation the mutation rate when they are finally mated is the same as it would have been after continuous mating. He concluded that mature sperm in unmated males is not stored but must be eliminated in some way. My own results, in a different stock--F<sub>1</sub> between two inbred lines Or and Erum 1--are in direct contradiction to this conclusion. In unmated males mature sperm accumulates, so that when they are mated the mutation rate is the mean for all sperm maturing up to that time. However, after 3-4 days' mating (at 3♀/♂/day), all previously matured sperm has been used up and the mutation rate settles down to that of males mated continuously since irradiation.

In confirmation of this it is simple to demonstrate that after a sterilizing dose of 30,000 r to a day-old male, all sperm which has passed the sensitive stage before irradiation persists and accumulates in the vesicula seminalis and vas deferens even when the testis proper has completely collapsed. When such a male is first mated, at whatever interval after irradiation, the viable sperm is utilized and a batch of eggs is laid with 100% dominant lethals. After that there is no more sperm, and no more eggs result from further matings.

Bateman, K. G. Studies in genetic assimilation.

Stocks showing various abnormalities of wing venation, occurring with frequencies of 90% - 100%, have been obtained by selection of flies in which the characters appeared spontaneously after a number of generations of phenocopy selection. The characters assimilated in this way are: absence of posterior crossvein, absence of anterior crossvein, presence of an extra crossvein in the first posterior cell, presence of an extra crossvein at the proximal end of the submarginal cell. A wild Edinburgh stock, S/W5, was used, but in addition an assimilated posterior crossveinless stock was obtained using Oregon-R. Further, a second stock of this character in the wild Edinburgh strain was obtained without phenocopy selection, by selection of rare crossveinless flies appearing spontaneously. Expression is variable in the extra-venation lines, but more or less constant in each of the crossveinless lines - being best in OR/R, where the vein is completely absent. There is some correlation between the two crossveinless and the two extra-vein types. Investigation of the genetic control of the characters is in progress now. There is already good evidence for a polygenic situation in all cases. Selection for phenocopy formation in an inbred line has so far been without effect.

Belitz, H. J. Ninyhydrin-positive substances in imagoes of *Drosophila*.

By means of two-dimensional paper chromatography, with phenol water and butanol-acetic acid water as solvents, the ninhydrin-positive substances of imagoes of *D. melanogaster* were studied. Flies were boiled for one minute in distilled water, decapitated, and then squashed at the start point. Less than half of the substances which could be found were free amino acids. These were: aspartic acid, glutamic acid, serine, threonine, arginine, tyrosine, valine (norvaline), and leucine (norleucine). It seems possible that by the use of other solvents some other amino acids could be found. For comparison, chromatograms were made of *D. funebris*, *repleta*, *hydei*, and *virilis*. No striking qualitative differences between these species and *D. m.* seem to exist, although probably there are some quantitative ones.

Bochnig, V. A determination of the oxygen consumption and the respiratory quotient of DDT-resistant and susceptible *D. melanogaster*.

From the susceptible "Berlin wild" stock a highly DDT-resistant strain was derived by permanent selection (DIS-26, p. 91). To establish whether the resistant strain differs physiologically from the susceptible one, oxygen consumption and respiratory quotient have been measured in unpoisoned flies of both strains and sexes by the Warburg method. The age of the examined imagoes was 1 to 48 hours. The  $O_2$  consumption was calculated per mg net weight and per fly. It was lower for resistant females than for susceptible ones in every case. The net weight is the same in females of both groups. Males of both strains showed the same values whether  $O_2$  consumption was calculated per mg net weight or per fly; they too have the same net weight. On the basis of net weight the  $O_2$  consumption of the males is the same as that of susceptible females, but because of their lower net weight they have a lower absolute  $O_2$  consumption than these females. The respiratory quotient is the same in all four groups. Its mean value is -0.83.

Braver, G., and Sandler, L. Failure to find sister-strand union in an attached-X chromosome.

Evidence of sister-strand union, which is a reverse attachment of sister chromatids, has been reported for heterochromatic sections of the X chromosome in inversion heterozygotes by Sandler (Genetics 39; in press). This event should usually lead to the production of a dicentric chromosome, which would be lost, and hence the event would, in most situations, be undetectable. In certain inversion heterozygotes, however, a recoverable product (a reversed acrocentric--double X--compound X chromosome) is produced if a normal euchromatic single exchange accompanies the sister-strand union.

Another situation in which this event would be detectable is that of an attached-X carrying distal heterochromatic segments. Sister-strand union in the distal heterochromatin plus a euchromatic single exchange would give rise to a reversed compound ring X chromosome (= double ring). In order to test for sister-strand union in this situation, the following experiment was performed. An attached-X was constructed composed of  $In(1)sc^{S1}$  car m w<sup>a</sup> y  $In(1)EN$  as one arm and  $y^{31d}$   $In(1)sc^8$  f v cv y  $In(1)EN$  as the other arm. Females carrying this attached-X and a Y chromosome were mated to B males. Sister-strand union in the distal heterochromatin (of either  $sc^8$  or  $sc^{S1}$ ) of this attached-X, if it were accompanied by a euchromatic single, would produce a female which would be phenotypically y (having lost both  $y^+$  and  $y^{31d}$  simultaneously). Such females would be viable, since the attached-X carries the y locus basally as well as

distally. The progeny from this cross included 26,836  $y^+$  or  $y^{3ld}$  females and one  $y\ v\ f$  female. This female was unfortunately semisterile (producing only 10 patroclinous males). The attached-X from which it was produced, however, appeared to be of the constitution  $y^{3ld}\ In(1)sc^8\ f\ m\ cv/In(1)sc^1\ car\ v\ w^a$ , and therefore the  $y\ v\ f$  female was most probably not simply a case of sister-strand union.

It might in addition be noted that if the distal heterochromatic sections paired in reverse order and a crossover occurred in this region, then a reversed compound ring X chromosome heterozygous for all of the markers heterozygous in the parental female would be produced. The experiment indicates that this would not be a feasible method of producing reversed rings (spontaneously, at least).

Brown, Wm. P., and A. E. Bell.

Minimizing differential survival in certain special linkage studies.

Among recombination classes except by progeny testing. The conventional method of introducing the unknown recessive mutant into a marker stock was not used for two reasons: (1) extensive progeny testing would be necessary to get da introduced into the marker stock; (2) recombination classes from backcross matings would still have to be progeny tested to distinguish da/da from da/+ females. After finding the approximate location of da with a dominant marker stock, B1 L, we made a more detailed linkage test by progeny testing individual females from the testcross Sp+J/+da ♀♀  $\times$  +da+/+da+ ♂♂. Recombination between Sp and J was naturally observed among the progeny of this three-point testcross; however, in order to distinguish between da/da and da/+ types in the testcross progeny, the individual female progeny test for sex ratio was necessary. Differential survival among the recombination types, especially those involving Sp, further complicated this approach.

In determining the locus of "daughterless" (da, see New Mutants), some difficulties were observed in that da/da zygotes as individuals are phenotypically wild type and da/da females could not be identified

The necessity of progeny testing to distinguish among the various recombination classes allows a new approach to the difficulty of differential survival of various mutants. Matings using recessive marker genes can be made in such a way that all individuals in the recombination classes are phenotypically wild type. They can then be separated into their appropriate recombination classes by individual progeny test. The following matings used in linkage studies with daughterless illustrate this method:

Parental--dp+b/dp+b ♀  $\times$  +da+/+da+ ♂

Backcross--dp+b/+da+ ♀  $\times$  +da+/+da+ ♂

Progeny test--8 recombination classes are phenotypically

wild type. Only ♀♀ can be used owing to nature of da. )  $\times$  dp+b/dp+b ♂

These ♀♀ are individually mated. )

Gross examination of offspring from the above progeny tests will separate those females into the various recombination classes for linkage calculations. Thus differential survival among recombination classes is minimized with no greater effort than using dominant marker stocks and less effort than introducing the unknown gene into a multiple recessive stock.

Burdette, Walter J. Penetrance of tumors in *Drosophila*.

Tumors occurring in susceptible strains of animals rarely appear in all individuals, but each strain usually has a characteristic

range of penetrance. Presumably, extrinsic factors are responsible, since this is true of both isogenic and inbred strains. Although rigid control of environment is not possible, it is possible to expose tumor stocks to the same changes in environment. This was done by duplicating culture conditions as nearly as possible and tabulating tumor incidence in eight different tumor strains twice each month at the same time for a period of six months. Fluctuations in environmental conditions presumably were the same for all cultures. In the tu <sup>36a</sup> vg bw and tu <sup>36a</sup> strains the incidence was impressively consistent throughout the entire period. In the others, however, changes in incidence were not the same either in direction or in extent, and no general seasonal variations were noted. When the stocks are arranged in order of susceptibility to tumors from highest to lowest incidence, or I to VIII in the accompanying table, the same sequence is not found on any two occasions. The number with tumors increased in some strains and decreased in others during the same period when incidence was unchanged in the remainder. This difference in response suggests a different mechanism of action for the tumor genes in the various strains, all of which have melanotic tumors that superficially are similar. (Aided by a grant from the National Cancer Institute, U.S. Department of Health, Education, and Welfare.)

Sequence (% Tumors)	Month during which tumors were counted (tabulations twice monthly)												*
	1a	1b	2a	2b	3a	3b	4a	4b	5a	5b	6a	6b	
(I)	8	8	8	8	8	8	8	8	8	8	8	8	
(II)	7	5	5	4	5	6	5	5	6	5	4	7	
(III)	6	7	6	6	7	7	6	4	5	6	6	2	
(IV)	5	6	7	2	6	4	7	6	7	2	7	5	
(V)	4	4	4	5	3	5	3	7	4	7	5	4	
(VI)	3	2	2	7	4	3	4	3	2	4	2	6	
(VII)	2	3	3	3	2	2	2	2	3	3	3	3	
(VIII)	1	1	1	1	1	1	1	1	1	1	1	1	

Strain Symbols: (1) tu <sup>36a</sup> (2) tu <sup>8</sup> (3) tu <sup>wps</sup> (4) y B <sup>263-43</sup> (5) tu <sup>48j</sup>  
 (6) se e <sup>11</sup> tu <sup>49h</sup> (7) vg mt <sup>A</sup> bw (8) tu vg bw

\* At least 1000 individuals from each strain counted per month.

Burdette, Walter J.  
 Heterochromatin and tumor  
 incidence.

The effect of variations in amount of heterochromatin within the cell on tumor incidence was tested by comparing the number of tumors in offspring

with different numbers of Y chromosomes. This was done by introducing the tandem X. Y chromosome obtained from Novitski into three different tumor stocks. Two types of crosses were made, mating w<sup>a</sup> B/X.Y females to X.Y males and the same type of females to males from the original tumor stock. The classes shown in the table which follows were then compared. The incidence in the mt<sup>A</sup> strain was remarkably uniform in all classes. However, there was variation in incidence between the different classes in the tu <sup>wps</sup> and tu <sup>48j</sup> strains, but the presence of the Y chromosome either alone or in tandem, singly or present twice, did not consistently either increase or decrease the number of individuals with tumors. No evidence was found, therefore, that the amount of heterochromatin present in the cell consistently alters the incidence of tumors in *Drosophila*.

Strain	Females				Males				
	$w^a B/+$	$+/X.Y$	$w^a B/X.Y$	$X.Y/X.Y$	$w^a B/0$	$0/X.Y$	$w^a B/Y$	$X.Y/Y$	
mt <sup>A</sup>	No.	<u>492</u> 716	<u>443</u> 664	<u>467</u> 624	<u>176</u> 255	<u>23</u> 39	<u>277</u> 443	<u>36</u> 54	<u>285</u> 453
	%	68.7	66.7	74.8	69.0	59.0	62.5	66.7	62.9
tu <sup>wps</sup>	No.	<u>82</u> 640	<u>99</u> 697	<u>34</u> 643	<u>25</u> 521	<u>8</u> 83	<u>15</u> 616	<u>21</u> 139	<u>46</u> 489
	%	12.8	14.2	5.3	4.8	9.6	2.4	15.1	9.4
tu <sup>48j</sup>	No.	<u>144<sup>a</sup></u> 456	<u>7<sup>b</sup></u> 450	<u>30</u> 684	<u>15</u> 139	<u>38</u> 236	<u>16</u> 629	<u>27</u> 174	<u>8</u> 423
	%	31.6	1.6	4.4	10.8	16.1	2.5	15.5	1.9

a:  $w^a B/ w^a B$ b:  $w^a B/X.Y$ 

(Aided by a grant from The National Cancer Institute, U.S. Department of Health, Education, and Welfare.)

Burdette, Walter J. Incidence of tumors in tu<sup>wps</sup> and tu<sup>49h</sup> strains with gt bb<sup>11</sup>.

In previous work, more tumors were found both in ligated larvae and in individuals with defective ring glands when the l(2)gl gene was introduced into tu<sup>wps</sup> and tu<sup>49h</sup>

tumor stocks. Subsequently and independently, Oster reported similar results for the tu<sup>6</sup> strain when gt was present. By appropriate crosses, we obtained sublines of both the tu<sup>wps</sup> and tu<sup>49h</sup> the strains having gt bb<sup>11</sup> heterozygous with  $w^a B$ . A comparison of tumor incidence in the four pertinent classes of adult offspring may be made from the table below. In the tu<sup>wps</sup> strain there was no convincing evidence of increase in number of tumors in gt bb<sup>11</sup>/Y males or gt bb<sup>11</sup>/gt bb<sup>11</sup> females in comparison to their siblings. On the other hand, the evidence for more tumors in tu<sup>49h</sup> flies with the giant genotype is much more convincing, particularly when only those hatching after the fourteenth day are used for comparison. The chief difference noted between tu<sup>wps</sup> and other tumors was that they usually appeared on the palps. This was also true in those with gt bb<sup>11</sup> homozygous or hemizygous, but when l(2)gl was present the tumors were found in the abdomen with few exceptions. At present, most evidence suggests that the humoral mechanism associated with metamorphosis accounts either primarily or secondarily for the regression of tumors in Drosophila. The apparent exception in the case of tu<sup>wps</sup> strain is not immediately explicable.

	tu <sup>wps</sup> (total)	Siblings			Giant Genotype			tu <sup>wps</sup> (hatched late)	tu <sup>49h</sup> (total)	tu <sup>49h</sup> (hatched late)			
		w <sup>a</sup> B/Y	w <sup>a</sup> B/gt	bb <sup>11</sup>	Total	gt	bb <sup>11</sup> /Y	gt	bb <sup>11</sup> /gt	bb <sup>11</sup>			
		No.											
	No.	14	307	17	308	31	615	5	230	18	227	23	457
	%	4.6		4.6		5.0		2.2		7.9		5.0	
	No.							1	24	2	34	3	58
	%							4.2		5.9		5.2	
	No.	17	66	26	278	43	344	47	269	52	153	99	422
	%	25.8		9.4		12.5		17.5		34.0		23.4	
	No.							24	36	22	26	46	62
	%							66.7		84.6		74.1	

(Aided by a grant from The National Cancer Institute, U.S. Department of Health, Education, and Welfare.)

Burdick, A. B., and Bell, A. E.  
Effect of medium pH on emergence time and egg production.

Crow's medium, which has a pH of ca. 4.0, was adjusted to pH's of 3.5, 4.5, 5.5, 6.5, and 7.5 (five bottles of each) with 3N HCl and 3N NaOH. Six previously mated pairs of

(CP x AP) F<sub>2</sub> flies were introduced without etherization to each of the 25 yeasted bottles and allowed to remain 48 hours. The bottles were incubated at a uniform temperature of 74° F, humidity of 50%, and in total darkness. Emerging adults were counted and removed every four hours over the 9-day period during which emergence took place. The bottles (1/2-pint creamers) yielded a mean of 440 adults per bottle. The accompanying table shows that, on the average, flies reared at 3.5 pH took about a day longer to emerge than at other pH levels.

Two strong frequency peaks of emergence took place at 197 hours and 220 hours of development. Twenty pairs were taken, without etherization, from each bottle at each of these times, and daily egg-production data were obtained on individual females for the second through the sixth day of adult life. This gave the mean daily egg-production data in the last two columns of the table. There are no significant differences in egg production of flies reared at different pH levels, but the 23-hour difference in "stage of emergence period" (220 hrs.-197 hrs.) is associated with a significant 11.2-eggs-per-day difference in production--the flies emerging 23 hours later producing fewer eggs.

It is quite surprising to find that such a variable trait as egg production, and one that is usually so responsive to environmental conditions during the laying period, is not affected by the drastic differences in rearing environment afforded by this experiment. Previous experience led us to expect the differences associated with time of emergence.

pH of medium	Total number of flies emerged	Mean hours to emerge	Average daily egg production of flies	
			emerging at 197 hrs.	220 hrs.
3.5	2463	236.1	89	76
4.5	1994	214.3	88	85
5.5	1891	213.6	95	79
6.5	2152	214.4	90	78
7.5	2523	215.9	95	83
Mean	440		91.40	80.20

Castiglioni, N. C. Further investigation of pseudotumors in *Drosophila*: experimental

The most recent experiments on reciprocal injections of hemolymph between tumorous and tumorless stock of *melanogaster* and *D. simulans* confirm the previous results.

*D. melanogaster*: Hemolymph from stocks with different incidences of pseudotumors is able to induce formation of the same with an incidence roughly proportional to the incidence of the donor stock. A stock of tu-white carrying pseudotumors with an incidence of 40%, induces pseudotumors in tumorless flies with an incidence of 16%; another tu-white carrying pseudotumors at 80% and tu-B3 at 84% induce pseudotumors in the same hosts with an incidence of 38% and 45%, respectively.

*D. melanogaster* and *D. simulans*: Hemolymph of a *simulans* stock with a high incidence of pseudotumors produces pseudotumors in the host (*melanogaster*), whereas the haemolymph of a normal *simulans* stock is unable to do so. In the latter case, however, the data are rather few; in fact, the mortality of the operated larvae is far higher than usual, probably owing to a certain incompatibility between *simulans* and *melanogaster* hemolymph.

At any rate, all these experiments seem to corroborate the hypothesis of an active participation of the hemolymph in the production of pseudotumors. This particular capacity of the hemolymph, moreover, seems to be related to the number of cells contained in the fluid: the hemolymph of a tumorous stock is richer in cells than that of a tumorless one. Preliminary observations permit one to distinguish the cells present in the hemolymph as large, medium, and small. Each of these classes includes some different cytological elements, which have not yet been definitively classified with regard to structure. A careful examination of these elements will be made. All types of large and medium cells were found in the hemolymph of both tumorous and tumorless larvae, with an evident prevalence of the larger ones in tumorous flies. Small cells

were found only in the tumorless larvae of one stock.

Counts were made on smears, using a fairly constant quantity of hemolymph; the stain used was May Grünwald Giemsa. The difference in number observed between two samples of hemolymph is of this magnitude: stock without pseudotumors--average number of cells, 500; stock with pseudotumors--average number of cells, 5000.

Edington, C. W., and Parker, D. R.  
Stock for detection and balancing  
sex-linked recessive lethal mu-  
tations in D. melanogaster.

In order to facilitate the detection of large numbers of spontaneous and induced sex-linked recessive lethal mutations in the  $F_2$  so that they could be easily maintained in stock, a modified Muller-5

stock was synthesized. In 1950 a Muller-5 male carrying a bobbed-lethal gene,  $bb^{150j}$ , which occurred spontaneously, was picked up. Males of this genotype were crossed to  $Df(1)N^8/In(1)dl-49$ ,  $y\text{Hw } m^2 g^4$  females and the  $F_1$   $Df(1)N^8/M-5$ ,  $bb^{150j}$  females were then backcrossed to their fathers. Since females homozygous for bobbed-lethal and  $Df(1)N^8$  males are inviable, a stock producing only  $Df(1)N^8/M-5$ ,  $bb^{150j}$  females and  $M-5$ ,  $bb^{150j}$  males can be maintained.

El Shatoury, H. H. A malignant tumor in *Drosophila*.

Among the sex-linked lethals induced by mutation workers in the laboratory, one ("lethal-malignant") has been found which

causes the development of tumors that exhibit metastasis and infiltration. The tumors originate from cells liberated from the lymph glands ("blood-forming organs") during the later part of the third instar. These move with the blood stream to the imaginal buds, which may become infiltrated. Mobile tumor cells may also pass along the ventral nerve cord, and infiltrate the fat bodies at the posterior end of the larva. Puparium formation is delayed from two to four days, and towards the end of this period processes antagonistic to the development of the tumor may clearly be seen. These take the form of various types of encapsulation of the tumor-mass, or of degeneration of the tumor cells to form melanotic masses rather similar to those previously described in other *Drosophila* "tumors", except that in lethal-malignant these masses are often attached to or actually inside the organs which have been infiltrated.

El Shatoury, H. H. The imaginal mesoderm in *Drosophila*.

A sex-linked lethal ("lethal no-differentiation") is characterized by the failure of some or all imaginal

buds to develop in the pupa. The expression is variable. The organs are affected in a definite order, the legs most commonly, sometimes the eyes in addition, sometimes the wings as well as the legs and eyes, then the thorax, and finally the genital disc. The defective development is due to overgrowth during late larval life of the imaginal bud mesoderm, which proliferates so as to disrupt the overlying epithelium of the bud. Just prior to the occurrence of this overgrowth, abnormalities can be seen in the lymph glands, in which certain cells degenerate. It seems probable that these cells normally secrete a hormone which holds in check the proliferation of the imaginal mesoderm; the order in which the primordia are affected would then indicate their responsiveness to the hormonal disturbance.

Epling, C., and Mattoni, R. H. T. Extension of the range of *D. miranda* (scarcely distinguishable by morphological criteria from *D. pseudoobscura*) has

been heretofore known to extend along the Pacific slope from Washington to central California. This range has been now substantially extended by the discovery of the species in a sample collected in March, 1954 at Keen Camp in the San Jacinto Mountains of southern California. This sample consisted of 61 cytologically determined flies of which 2 males and 5 females were D. miranda.

One wonders about the interpretation of this new record. The population at Keen Camp has been under almost continuous observation since 1939, and the chromosomes of approximately 7000 individuals of D. pseudoobscura have been determined for inversions of the third chromosome. The hybrids between D. miranda and D. pseudoobscura, as well as the species homozygotes, have a characteristic chromosome pattern in the salivary glands; thus, the presence of D. miranda would not be easily passed by during routine determinations. The March collection, however, is unique for this station on account of weather. Associated with this fact is that no D. miranda were found in later collections this year from the same and adjacent stations.

Two explanations are possible. First, that the species has been transported southward during recent times. This explanation receives support from recent additions to the inversion types previously known elsewhere and now present in this population. They, as well as D. miranda, may be adventive. The second possibility, however, is that the species has existed heretofore at higher elevations, and that it is most abundant during the winter months, which would be in keeping with its seasonal requirements elsewhere, and exists only at very low frequencies during the summer when most sampling is necessarily done. This explanation received support from the fact that the cross between the San Jacinto strains and one from the Olympic peninsula, kindly supplied by A. H. Sturtevant, differs by at least one inversion that is found in the X2 chromosome, which is known to be partly homologous with the third of D. pseudoobscura. The heterozygote resembles the ST/AR inversion of D. pseudoobscura in position and length. There is no assurance, however, that this inversion does not occur to the north.

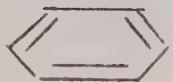
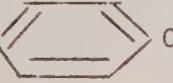
Fahmy, O. G., and Fahmy, Myrtle, J. The mutagenicity of the amino acid mustards in D. melanogaster.

It has been reported (DIS-27) that the mustard derivative of phenylalanine, p-di-(2-chloroethyl)-amino-phenylalanine, is a strong mutagenic agent when injected around the testes of adult *Drosophila* males.

Several attempts have since been undertaken to investigate the effects of minor changes in the chemical structure of this compound on its mutagenic properties. A mustard derivative of a higher analogue of phenylalanine, viz., p-di-(2-chloroethyl)-amino- $\gamma$ -phenyl- $\alpha$ -amino-butyric acid, has therefore been tested for mutagenic activity. Equal concentrations of the two compounds were used and they were injected in males of equal size and age. In both cases the mutation rate was determined by the Muller-5 test for sex-linked recessive lethals among the progeny produced three days after treatment. The structural formulae for the compounds used and the mutation rates they induced at a concentration of 0.25% are given in Table I.

Table I

Sex-linked recessive lethals induced by equal concentrations (0.25%) of mustard derivatives of related amino acids

Compound		No. of chromosomes	No. of lethals	% lethals
(Cl CH <sub>2</sub> CH <sub>2</sub> N  CH <sub>2</sub> CH <sub>2</sub> NH <sub>2</sub> ) <sub>2</sub>	CH <sub>2</sub> CH COOH   NH <sub>2</sub>	1147	36	3.1
p-di(2-chloroethyl)-amino-phenylalanine				
(Cl CH <sub>2</sub> CH <sub>2</sub> ) <sub>2</sub> N  CH <sub>2</sub> CH <sub>2</sub> CH COOH   NH <sub>2</sub>		219	6	2.7
p-di(2-chloroethyl)-amino- $\gamma$ -phenyl-d-amino-butyric acid				

The data suggest that there is no significant difference between the mutation rates induced by the two amino acid mustards tested. It should be noted that phenylalanine is a naturally occurring amino acid whereas its higher analogue is not. This difference does not seem to be significant as regards the mutagenic activity of their mustard derivatives.

Table II

Dominant lethals induced by phenylalanine mustard: the L & D isomers and their mixture

Dose & compound	DOMINANT LETHALS					
	Mixture		L		D	
	No. of eggs	Unhatched	No. of eggs	Unhatched	No. of eggs	Unhatched
0.5	8037	75.9	6591	80.1	1931	87.2
0.3	13089	56.3	16901	65.5	9708	74.4
0.15	23242	30.6	21608	45.6	12096	50.7

Table 3

Sex-linked recessive lethals and visibles induced by phenylalanine mustard: the L & D isomers and their mixture

Dose % compound	Mixture				L				D			
	Chr. tested	% lethal	% vis.									
0.5	1207	9.8	1.8	345	8.4	3.5	198	10.6	4.0			
0.3	1916	6.0	2.3	1438	9.1	2.0	399	8.3	2.5			
0.25	-	-	-	1763	6.0	1.5	1599	4.6	1.1			
0.15	2111	3.0	1.0	-	-	-	-	-	-			

The effect of the optical configuration of the amino acid part of the mustard molecule on mutagenicity has also been investigated. The biological activity of the mustard derivatives of the D & L isomers of phenylalanine, as well as the mixture, were compared as regards the rate of induction of dominant lethals (Table 2) and sex-linked recessive lethals and visibles (Table 3) scored in the same Muller-5 experiments. As regards the dominant lethals the results are consistent, and they suggest that the D-form is more active than the L-form at all concentrations, the mixture having less activity than either. For the recessive lethals and visibles, on the other hand, there seem to be no clearly defined differences.

Falk, R. Nonstability of stocks with the y f:= chromosome.

It is known that in ordinary attached-X females there occurs a small proportion of detachments. The double-attached y f:= of Muller is more practical to use as a balancer of sex-linked genes in males, as it is more stable (Muller, 1943, DIS-17: 61). While using double-attached females, however, there appeared some females that were not maternal with regard to phenotype.

Two daughters of the cross y f:=/sc<sup>8</sup> Y x B car/sc<sup>8</sup> Y were analyzed. They showed a slight Bar effect and one was also forked; and they were found to be triploids. During the analysis at least two more triploid females were found, and two females that were apparently superfemales. In these triploid females the crossing over frequency was rather high even in the vicinity of the inversions (Valencia et al., 1949, DIS-23: 99-102), and all four triploids had among their progeny crossover products that involved the free X chromosome.

The crossovers were: (1) Between the free X chromosome and the distal of the double-attached X's in the interval from f to dl-49, so that a f:= chromosome and a y w dl-49 B car chromosome were obtained. (2) Between the proximal arm of the double-attached X's and a M-5 chromosome, which in this case was the free chromosome, from which a y w<sup>a</sup> f:= chromosome was obtained (this chromosome has the centromere of the M-5 chromosome and is a compound w/w<sup>a</sup>; the fly was yellow because it contained yellow in the distal and yellow deficiency in the proximal X). (3) A double crossover between the proximal of the double-attached X's and a free M-5ry chromosome (a M-5 chromosome with a yellow mutation but without Bar). One crossing over occurred in the interval between w<sup>a</sup> and Ins, the other between f and y. This female was a y:= (it had dl-49 in one arm and

INS in the other).

No counts were made of the appearance of exceptional daughters of double-attached females, but the frequency in the stocks seems to be higher than 1:1000. Even if most of them are superfemales and therefore sterile, care must be taken to avoid loss of the desired X chromosome through triploid females.

Farnsworth, M. W. On obtaining a haplo-IV stock.

From a stock obtained from Dr. E. Novitski and originally described by Lindsley and Novitski (DIS-27: 99), it has been possible to extract a haplo-IV strain of D. melanogaster.

Males of the stock y w. IV/YL?/y<sup>2</sup> suwa wa bb were crossed to Canton females, and the resulting  $F_1$  female progeny were backcrossed by pair matings to Canton wild-type males. Of the 21,336  $F_2$  progeny, 96, or 0.22%, were Minute in phenotype and were tentatively considered to be haploid for the fourth chromosome owing to non-disjunction of this chromosome during meiosis. Of these 96 Minute progeny, 42 were females, + Minute in phenotype, 28 were + Minute males, and 26 were y w Minute males. These individuals were crossed singly to Canton wild-type flies, and of the many cultures prepared only those in which + Minute males were used produced any second-generation Minute progeny. From these, several lines were isolated, two of which are being maintained at present by continuous backcrossing to Canton. Study of aceto-orcein squash preparations of larval brains and salivary glands from one such line has definitely shown it to be haploid for the fourth chromosome. Although 50% is the expected frequency of haplo-IV flies in any one backcross generation, usually less than 10% of the progeny are Minute, the males outnumbering the females. The many non-eclosed pupae remaining in culture vials and the very small size of imaginal discs in dissected larvae would indicate that many haplo-IV individuals fail to complete pupation. In crosses with ci ey<sup>R</sup> flies, where the  $F_1$  classes ci ey<sup>R</sup>/+ and ci ey<sup>R</sup>/0 (haplo-IV) are expected, only the former eclose, the ci ey<sup>R</sup>/0 individuals being represented by non-eclosed pupae. Examination of such pupae has revealed that the thorax, abdomen, and appendages are normal, but that the head is represented only by a small knob with a few hairy protuberances. The labellae of the mouthparts, however, are usually intact but reduced in size. All such individuals have Minute-type bristles and evidently make up the missing class of progeny. The exaggerated expression of ey<sup>R</sup> is to be expected in view of the reported effect of a mutant in combination with a deficiency for the same locus.

It should be noted that sterility is common among adult haplo-IV males and is even more pronounced in adult females, although successful inbreeding has been carried out.

Freire-Maia, N. Pericentric inversions in Drosophila.  
summarized as follows:

The present situation regarding the pericentric inversions found in Brazilian populations of D. ananassae may be summarized as follows:

Localities	Region	Number of individuals examined	Time of collection	Pericentric inversions	No. of times
Passagem, Pr	South	54	June, 1951	A	1
Recife, Pe	North	35	July, 1951	B	1
Antonina, Pr	South	62	March, 1952	C	2
Antonina, Pr	South	25	November, 1951	-	0

Localities	Region	Number of individuals examined	Time of collection	Pericentric inversions	No. of times
Paranaguá, PR	South	67	September, 1952	D	1
Paranaguá, PR	South	46	March, 1952	-	0
Paranaguá, PR	South	49	October, 1953	C	2
Paranaguá, PR	South	29	September, 1954	-	0
Uberlândia, MG	Center	29	March, 1953	E	1
Uberlândia, MG	Center	30	March, 1954	-	0
Others	North, Center and South	798	- - -	-	0
TOTAL		1224		5	8 (0.65%)

As pointed out in our previous note (DIS-27: 91-92), the number of different pericentric inversions found in natural populations of *D. ananassae* is higher than that detected, in the same conditions, in all the other *Drosophila* species taken together. It is noteworthy that one of these inversions (C) has been found in two different localities of the southern border of Paranaguá Bay (Antonina and Paranaguá), about 25 km apart in a straight line on the Bay.

Freire-Maia, N.,  
Freire-Maia, A., and  
Beltrami, W. M. T. The Hardy-  
 Weinberg Law in Brazilian  
 natural populations of *D.*  
kikkawai.

Natural populations of *D. kikkawai* Burla, 1954 (previously reported in some of the senior author's papers as *D. montium* de Meijere, 1916) are polymorphic with regard to the intensity and extension of the pigmentation in the abdominal tergites. Two homozygous forms (a dark and a light one)

have been isolated, and a series of crosses showed that the allele for light is recessive. Both forms have been found in various Brazilian populations, the light phenotype being always commoner than the dark one. The light form, however, is less common in the southern localities (frequency, 64.55%) than in the others (frequency, 83.04%), as can be clearly seen in the following table, where the names of the localities appear in the order they occupy from north to south. This difference is statistically significant (chi-square equals 4.54).

"Northern" localities	Females			Light females (%)
	Light	Dark	Total	
Goiânia, GO	21	4	25	84.00
Belo Horizonte, MG	21	5	26	80.77
Lins, SP	41	5	46	89.13
Assis, SP	1	0	1	100.00
São Paulo, SP	245	62	307	79.80
Londrina, PR	17	3	20	85.00
Antonina, PR	21	4	25	84.00
Morrêtes, PR	240	41	281	85.41
TOTAL	607	124	731	83.04
<u>"Southern" localities</u>				
Paranaguá, PR	134	72	206	65.05
Ascurra, SC	11	1	12	91.67
Itajaí, SC	43	29	72	59.72
Gaspar, SC	11	9	20	55.00
Florianópolis, SC	18	11	29	62.07
Porto Alegre, RGS	5	0	5	100.00
TOTAL	222	122	344	64.53

It is interesting to note that the southern group of localities begins in Paranaguá, a locality just about 30 km distant from Antonina and Morrêtes, which present a typically "northern" genetic composition. The following data for Morrêtes and Paranaguá, obtained with females collected on the same days show that this difference is highly significant (chi-square equals 15.95):

Localities	Females			Light females (%)
	Light	Dark	Total	
Morrêtes	122	20	142	85.92
Paranaguá	35	23	58	60.34

As the allele for dark pigmentation is dominant over that for light, it is impossible to detect the genetic composition of the samples through direct microscope examination of the flies. The crosses we made between collected males and light females from laboratory strains revealed, as was previously found by the senior author in artificial populations kept in the laboratory, that the three genotypes were always present in accordance with the Hardy-Weinberg formula. The following table with data from Morrêtes (April, 1954) and Itajai (July, 1954) shows two examples of this situation:

Localities	Homozygous	Heterozygous	Light	Total	Chi-
	dark (AA)	dark (Aa)	(aa)		square
Morrêtes	0	6	62	68	0.142
	0.13	5.74	62.13	68	
Itajai	0	18	31	49	2.475
	1.65	14.70	32.65	49	

The homozygous dark genotype is relatively rare, being found just once in the total sample of 301 males examined by this method. Analysis of the offspring of collected light females inseminated in the natural populations revealed the same fact. The heterozygous were always present, however, with positive--although insignificant--deviations.

Fung, Sui-Tong Chan, and Gowen,  
John W. Gonad development of  
hermaphrodites (Hr) in D.  
melanogaster.

The Hermaphrodite gene (Hr) in the third chromosome aggects normal diploid females, giving rise to individuals with a mixture of male and female sexual organs (Gowen, 1942). Histological observations of the

different larval and pupal stages have been made. The hermaphrodites can be distinguished from the normal males and females as early as the second-instar larval period. Normal female gonads contain small, compact cells of irregular arrangement. The male gonads are large and the spermatogonial cells are uniform in size. The hermaphroditic gonads have two types of cells: small cells of oögonial-like type located at the two ends; and larger cells, usually in a cluster of 8 or 32, found in the central portion enclosed by the fibrous-like tissue. These giant cells are dense in cytoplasm, have large nuclei, and seem to contain chromosomes in polytene condition. Such features are recognizable until the late pupal stage. Their significance to sexual development is not clear. In the adult stage, the gonads are generally rudimentary, but in some cases they may attain mature size. Gonads develop toward the ovarian type, with quite a range in the stage at which development stops. Not a single case has yet been observed which shows the development of the hermaphroditic gonad into an adult testis. Yellow pigment characteristic of the testicular external epithelium is present in the vasa efferentia, and the pigmentation occasionally also extends as a granular coating over the rudimen-

tary ovary. These findings correlate with those of our transplantation experiments, in which male genital discs were introduced into normal females and hermaphroditic genital discs into normal females. In the first of these, the vasa of the transplants developed yellow pigment in the female hosts. In the second case, the vasa of the transplants as well as the hosts' ovaries developed tiny masses of yellow pigment. Pigment production in these cases, even though a normal testis is absent, suggests that the vasa acts as an inductor under whose influence the ovary can produce pigment.

Green, M. M. A 3N stock which requires no selection.

stock has been made up in which only the 3N females are fertile and no selection is required. In this stock the 3N females have attached-X chromosomes homozygous  $ct^h$   $sn^c$ , and a free X chromosome,  $scS1$   $d1-49$   $v$ , whereas the males are  $scS1$   $d1-49$   $v$ . Since  $sn^c$  and  $scS1$   $d1-49$   $v$  are homozygous female sterile, only the 3N females are fertile and the stock maintains itself in this way with the  $scS1$   $d1-49$  inversion preventing crossing over between the attached X's and the free X chromosome.

Haddox, C. H., Jr. Tumors in natural populations of Drosophila.

Wild Drosophila were collected during the months of April and May, 1954, from several areas in and near the city of New Orleans (none nearer than 2000 meters from the tumor stocks at Louisiana State University), in a search for flies bearing hereditary tumors. Only two flies with tumors were found, both male, and they were found to be sterile. The progeny from each female was examined. There were 136 D. melanogaster females which were fertile and produced 4578 offspring, and 130 D. simulans produced 5011  $F_1$  progeny. Among 173  $F_1$  from 4 original D. melanogaster females, 6 individuals with tumors were found. Tumors appeared in 36 offspring among 851 progeny of 17 original D. simulans females. Utilizing crosses to  $w^a$   $B$ ;  $Pm/Cy$ ;  $H/Sb$ , an average of 1331 individuals per line were examined over six generations. It was possible to recover only two lines in which tumors were repeatedly found. In one the incidence is only 0.44% (10/2291), but it is 12.2% (76/622) in the other. The former is D. melanogaster, the latter D. simulans (see New Mutants). Failure to recover tumors in the remaining lines could be due to loss of tumor gene(s), low penetrance, or nonhereditary nature of the abnormality. Caution should be exercised in scoring Drosophila for tumors. In these studies a number of individuals at first thought to have tumors were found to be injured or infected, or to have ingested pigmented material.

Species	Female	Male	Both	No. tumors	Per cent tumors
<u>D. affinis</u>	4	9	13		
<u>D. hydei</u>	16	34	50		
<u>D. immigrans</u>	2	3	5		
<u>D. melanogaster</u>	411	383	794	2	0.3
<u>D. putrida</u>	0	1	1		
<u>D. repleta</u>	10	14	24		
<u>D. simulans</u>	493	481	974		
<u>D. tripunctata</u>	6	1	7		
<u>D. virilis-texana</u>	7	8	15		
Unidentified	0	1	1		
<b>TOTAL</b>	<b>949</b>	<b>935</b>	<b>1884</b>	<b>2</b>	<b>0.1</b>

Hadorn, E. Transitory appearance of a fluorescent substance in eye-color mutants of D. melanogaster.

Hadorn and Mitchell (Proc. Nat. Acad. Sci. 37, 1951) have shown that eye-color mutants, such as white and brown, not only affect the visible pigments but also reduce a series of fluorescent substances. One

of these compounds, which we called "Fl-3," deserves special attention. This substance shows an intense violet-blue fluorescence. According to the unpublished work of my colleague Professor M. Viscontini (Chemisches Institut der Universität Zürich), it is very probably identical with iso-xanthopterine. In the wild type, the synthesis of "Fl-3" begins in the third larval instar. Then during the first half of pupal life this substance accumulates considerably, reaching a final concentration which is maintained during imaginal life. Since the testes contain great quantities of Fl-3, we find much more of this substance in males than in females. Fl-3 was found to be completely absent in adult and bw flies of at least 3 days of age. Recent studies have shown, however, that this deficiency is not due to a gene-conditioned block in a synthetic process. Larvae and young w and bw pupae do accumulate Fl-3 at first. A maximum concentration, which is lower than in the wild type, is reached at the same time as in wild pupae. Then the concentration decreases steadily during the period covering the end phase of metamorphosis and the first two days of imaginal life, until none of this substance is left in the bodies of w and bw flies.

Harrison, B. J. HCN resistance in D. melanogaster.

Wild flies caught at Bayfordbury have been subjected to concentrations of HCN that originally gave a 90% (C.T.P. 0.28) and 50% (C.T.P. 0.15) kill. (Percentages are sex means; females are slightly more resistant in the Bayfordbury stock.) Resistant flies were selected and the concentration-time products of 0.28 and 0.15 repeated. An unselected line of Bayfordbury wild type was continued on a large scale to preserve the original variability. The unselected line has remained around the 10% recovery point (at C.T.P. 0.28) for 46 generations. Two distinct lines were selected at C.T.P. 0.28, and both have gradually risen until after 46 generations they both exhibit a recovery of 75%-80%. When these two lines were crossed, no increase in resistance was shown. Resistance was built up more rapidly at the higher C.T.P.

Chromosome assays (using Cy L<sup>4</sup>/Pm; H/Sb as markers) have shown that all or nearly all the resistance in both 0.28 lines was located on the second chromosome (fourth chromosome untested). Kikkawa, Ogaki, and Tsukamoto (DIS-27) and Dresden and Oppenworth (DIS-27) also obtained lines resistant to DDT, BHC, HCCH, and other insecticides; and they also found that the resistance was on the second chromosome. Through the kind cooperation of these investigators, the various resistant stocks were mutually exchanged and tested for cross-resistance. No cross-resistance occurred in any of the tests with HCN. The gene or genes responsible for HCN resistance are therefore dissimilar from the genes governing resistance to the other insecticides tested, but are located on the same chromosome.

Harrison, B. J. Selection for differences in chaeta number between the fifth and fourth sternites of D. melanogaster.

which possessed more chaetae on the fifth than on the fourth were selected and

Twenty generations of selection were made on Samarkand (inbred for 107 generations) x Oregon (inbred 312 generations) for differences between the chaeta numbers of the fifth and fourth sternites. Individuals

continued by 2 x 2 matings. Similarly, those that showed more chaetae on the fourth than on the fifth were selected. A control line was established in which flies were selected which showed no difference between the two sternites. No increase in sternite difference occurred in 20 generations. The gross chaeta number of the two sternites showed an increase in the two lines selected for identity. These increases were probably results of chance selections for high chaeta number. Differences between the numbers of chaetae on the sternites were not related to the gross number.

Mather (Heredity 7: 297-336, 1953) obtained asymmetrical differences in sternopleural chaetae within 20 generations of selection, using similar material. It would seem, therefore, that responses to selection for longitudinal differences are less easy to obtain or observe than bilateral. This may be due to differences in expression or to different genetic correlations. The correlation between sternites appears to be high. The fact that the gross number of chaetae on the fifth and fourth sternites is amenable to selection shows that there is genetic heterogeneity for this character, and the high correlation between the sternites means that these genetic differences have similar effects on both sternites.

Harrison, B. J. X-irradiation and selection.

X-irradiation of 4000 r was given every generation to selected males prior to mating to unirradiated selected females, and the

responses to selection were compared with completely unirradiated selections. The selections were for low and high abdominal chaetae on Samarkand inbred, Samarkand mass, and Samarkand inbred x Oregon inbred (2 x 2 matings).

Samarkand (inbred 95 generations):

40 generations of high selection, irradiated--No increase in chaetae number.  
 40 generations of high selection, unirradiated--No increase in chaetae number.  
 20 generations of low selection, irradiated--Significant decrease in chaetae at S10 (i.e., 10 generations of selection). Further decrease at S17, then line became infertile.  
 21 generations of low selection, unirradiated--Significant decrease at S13. Further decrease at S18, and line failed through infertility.

Repeate Selection (Samarkand inbred 147 generations):

10 generations of high selection, irradiated--No responses.  
 10 generations of high selection, unirradiated--No responses.  
 10 generations of low selection, irradiated--No responses.  
 10 generations of low selection, unirradiated--No responses.

Samarkand Mass:

40 generations of high selection, irradiated--A considerable response at S12 owing to wing which affected chaeta number pleiotropically. Very high variance.  
 40 generations of high selection, unirradiated--No response.  
 40 generations of low selection, irradiated ) Both responded, the irradiated  
 40 generations of low selection, unirradiated ) line a few generations earlier,  
 i.e., at S10; unirradiated at S14. (Irradiated low selection very infertile after S15).

Samarkand (inbred 147 generations) x Oregon (inbred 541 generations):

34 generations of high selection, irradiated--No response until S20. An unir-

radiated subline taken off at S20; both then onwards responded moderately and equally.

34 generations of high selection, unirradiated--Slow response from S2-S13 and then stable until S34, when line was terminated.

10 generations of low selection, irradiated ) Both responded slightly and 10 generations of low selection, unirradiated ) equally.

Samarkand (inbred, 181 generations) x Oregon (inbred 383 generations):

6 lines of high selection, irradiated for 7 generations ) A slight response  
6 lines of high selection, unirradiated for 7 generations) in some lines, but when pooled there is no difference between irradiated and unirradiated selections.

As irradiation was given only to males, little or no effect upon recombination would have resulted. The failure of response in numerous lines tested concurrently, together with the results obtained from the less extensive tests, shows that mutation has no significant effect at the polygenic level, either (1) because polygenes are less mutable than major genes, or (2) because the effects of the mutations are too small to be selected. The only significant increase in chaeta number was when a major wing mutation affected the chaetae pleiotropically. A stimulus which increases the rate of recombination is therefore more likely to produce the release of variability from which responses to selection might be obtained.

Hinton, Claude W. The occurrence of fragments of the unstable  $w^{vc}$  chromosome.

A group of 31 fragments derived from the unstable  $In(1)X^{c2}$ ,  $w^{vc}$  ring chromosome have been recovered from crosses involving  $w^{vc}$  and a variety of y attached-X or rod chromosomes.

Of the total, 26 of these fragments were observed as  $y^+$  male tissue in sterile gynandromorphs or  $X0$  males which arose by elimination of  $w^{vc}$  from  $w^{vc}/y$  zygotes. Five other fragments were recovered in  $\bar{X}X$  females and a comparative analysis of these has been initiated. All 5 are unstable, although in obviously different degrees, and all of them appear to be small rings in neuroblast preparations. With reference to constitution, all 31 cases covered y, 9 of 15 diagnostic cases covered w, and 2 of 10 diagnostic cases covered spl. No mutant locus to the right of spl was found to be covered by any fragment, although a test was possible in one or more cases for the mutants  $sn^3$ ,  $lz^s$ ,  $m^2$ , f, B,  $g^4$ , and car.

Fifteen of the fragments found in gynandromorphs or in  $X0$  males were present in only part of the male tissue. This observation, plus the instability of the 5 fragments recovered in  $\bar{X}X$  females, shows that the instability of the entire  $w^{vc}$  chromosome must be a property of some region of the chromosome common to all the fragments, namely, the centromere, the proximal heterochromatin, and the short distal euchromatic region y-w. It is possible to consider both the production of  $w^{vc}$  fragments and  $w^{vc}$  elimination as consequences of the formation of double anaphase bridges by the  $w^{vc}$  chromosome during somatic mitoses.

Kanehisa, T. Some aspects of maternal effect on the expression of tumors in D. virilis.

Studies were carried out on the following two questions: (1) whether tumor development is associated with infection; and (2) whether the maternal effect on tumor incidence is continuous through later generations. The flies used in the experiments were derived from a tumorous stock showing a tumor incidence of about

62%. The following investigations were made to examine the first question: (a) culture of tumor and nontumor eggs in the same bottle; (b) infection of tumor from tumor imago to nontumor eggs, larvae, or pupae; (c) injection of tumor extracts into a nontumor larva. As far as the experiments have gone, they have failed to prove that tumors occur by infection. As to the second question, all the available evidence seems to indicate that maternal effect on tumor incidence continues through successive generations in various matings, whether the parents are tumor mothers or fathers. The offspring are high in tumor expression when the mothers show high tumor expression. On this basis it may be assumed that tumor expression is regulated by a cytoplasmic factor (or factors).

Kikkawa, H., and Fujito, S. The chemical nature of resistance to insecticides.

As described earlier, resistance to DDT, BHC, and parathion seems to be controlled by a dominant gene located near locus 66 in the second chromosome. We have examined the chemical nature of this resistant gene, and found that it is closely related to the strength of specific cholinesterase. Thus, the strength of cholinesterase in resistant strains like Hikone and Kochi is about twice that of nonresistant strains like Canton-S and Sendai. Furthermore, it has recently been found that flies of resistant strains contain an abundance of Fe as compared with flies of nonresistant strains. These facts may give a clue to the solution of this problem.

Kikkawa, H., Ogita, Z., and Fujito, S. Inborn capacity of absorption of metals in *Drosophila* mutants.

Recently it was found that brown pigments present in *Drosophila* eyes are composed of metallic complex salts involving iron, copper, cobalt, etc. (Kikkawa, Ogita, and Fujito, Proc. Japan Acad. 30: 30, 1954). This concept has been extended to include pigments that are assumed to be derived from tyrosine (Kikkawa et al, Science, in press). There is a relation between coloration and kind of metal, that is, white is associated with Ni, yellow with Ti, red with Mo, and blackish with Cu, Co, and Fe. Thus, in *Drosophila*, ebony or black mutants contain large amounts of Cu, Co, and Fe, whereas yellow or tan mutants have Ti in abundance.

Our experiments showed that different eye- or body-color mutants have a special ability to absorb certain kinds of metal even if they are bred on the same medium. It is of interest that when wild, yellow, and ebony larvae are bred on medium containing Cu, ebony cannot survive, whereas on medium containing Ti, yellow and wild die and only ebony can survive. This phenomenon is always recognized among mutants absorbing different kinds of metals. For reference purposes, we show a tentative map concerning this problem.

Chromosome 1

yellow (Ti)  
white (Ni)  
eosin (Cu)  
apricot (Fe)  
ruby (Co)  
carmin (Fe, Ni)  
tan (Ti, Ni)  
vermilion (Fe, Ni)  
garnet (Co)  
carnation (Fe, Ni)

Chromosome 2

clot (Ti, Ni)  
black (Cu, Co, Fe)  
purple (Cu, Co)  
straw (Cu, Ni)\*  
cinnabar (Co)  
brown (Co)

Chromosome 3

sepia (Ti, Ni)  
scarlet (Cu, Ni)  
karmoisin (Cu, Ni)  
ebony (Fe, Cu, Co)  
cardinal (Fe, Ni)  
claret (Cu, Fe)

\* Straw is an exceptional case. In spite of its yellow body color, this mutant does not have Ti. This may be due to the decrease of melanin pigment.

Koref, Susie, and Brncic, Danko. A study of the chromatographic patterns in tum oral and nontumoral larvae of D. melanogaster.

Hadorn and Mitchell (1951) and Buzzati-Traverso (1953) studied the chromatographic patterns of D. melanogaster. The latter found that each genotype had a specific biochemical pattern, which was highly independent of diet and surrounding conditions.

This work led the authors to study the

biochemical patterns of tumorous and nontumorous larvae of two stocks, w<sup>a</sup> B tu<sup>48j</sup> and se ell tu<sup>49h</sup>. Larvae of the nontumorous stocks w<sup>a</sup> B and se ell, with a residual genotype similar to that of the above-named stocks, were used as controls. The ascending chromatographic technique described by Hadorn and Mitchell was used. Ninety-six-hour-old larvae of the six groups were squashed separately on a sheet (23 x 15 cm) of Whatman No.1 paper. This sheet was placed in a solvent consisting of distilled water, butanol-n, and glacial acetic acid. The spots were revealed under ultraviolet light and by ninhydrin (0.25% solution in acetone).

With ninhydrin, 6 spots were observed. These were the same in all the larvae examined. Under ultraviolet light, 7 spots were seen. Five of them were the same in the six groups of larvae; the other two--one brilliant yellow (Rf: 0.380), the other lilac (Rf: 0.465)--could be clearly distinguished in the se ell and in the tu<sup>49h</sup> larvae, with and without tumors. These spots were practically absent in the w<sup>a</sup> B and tu<sup>48j</sup> larvae. These results were very constant in 126 chromatograms analyzed, and indicated that: (1) There are no visible chromatographic differences between the tumorous and nontumorous larvae, using these methods. (2) During the larval stage there are clear and constant biochemical differences between individuals of different genotypes.

Koref, Susie, and Brncic, Danko. Effect of residual genotype on expression of tumoral genes in three tumoral stocks of D. melanogaster.

nontumorous stocks: Oregon R-C, w, f, dp, bw, st, se, ey<sup>2</sup>. The number of tumors observed in the second generation was variable.

Three series of experiments were performed to study the effect of the residual genotype on the expression of the tumorous genes in three different stocks: tu vg bw (99.86% tumors), w<sup>a</sup> B tu<sup>48j</sup> (48.3%), se ell tu<sup>49h</sup> (63.2%). Each of these stocks was crossed with eight different

stocks was crossed with eight different

In the first series of crosses with tu<sup>48j</sup>, 135 out of a total of 14,649 flies had tumors. There was a marked difference between the number of tumors in the different crosses. Comparing each of them to tu<sup>48j</sup>/Oregon R-C (0.48% tumors), considered the control group, the differences were statistically significant in the crosses with dp (1.4% tumors) and with se (8.02% tu). In the second series, se ell tu<sup>49h</sup>, out of 16,152 flies, 1,166 had tumors. Comparing again with the cross tu<sup>49h</sup>/Oregon R-C (2.81% tumors), the matings with bw (8.44% tu), st (5.07%), and se (29.17% tu) had a significantly higher incidence. In the third series, tu vg bw, 854 out of 16,376 flies had tumors. The crosses with f (1%), dp (16.73%), and se (12.13%) had significantly different tumor frequencies.

It can be concluded from these results that the expression of tumors depends greatly on the structure of the residual genotype in which the principal tumor genes are found. In some cases it stimulates tumor growth, whereas in others it inhibits it. The genotype se was highly stimulating to neoplastic growth in all three cases. These results prove that the tumoral genes, of

incomplete expression and penetrance, are influenced not only by changes in the external surroundings but also by modifications of the genetic structure in which they are found.

Kuroda, Y. Tissue culture  
of eye discs of D. melanogaster.

Newly laid eggs were sterilized with a 0.05% HgCl<sub>2</sub> solution of 70% ethyl alcohol for 30 minutes, then transferred to sterilized bottles containing food. Third-instar larvae grown under such sterile conditions were dissected under a binocular microscope in sterilized Ringer's solution. The eye discs were cultivated in hanging drops by means of the cover-slip method. The components of the synthetic medium used for cultivation were as follows:

Substances	mg/l	Substances	mg/l
Casein hydrolyzate	50,000.0	Folic acid	0.05
Tryptophane	1,000.0	Choline hydrochloride	2.5
Cystine	1,000.0	Inosite	0.25
Adenine hydrochloride	50.0	p-Aminobenzoic acid	0.25
Guanine hydrochloride	1.5	Vitamin A	0.5
Thymine	1.5	Vitamin B <sub>12</sub>	0.5
Uracil	1.5	Ascorbic acid	0.25
Xanthine	1.5	Glucose	800.0
Thiamine	0.05	NaCl	7,000.0
Riboflavin	0.05	KCl	200.0
Pyridoxine hydrochloride	0.125	CaCl <sub>2</sub>	20.0
Niacinamide	0.125	MgCl <sub>2</sub>	100.00
Ca-pantothenate	0.05	NaHCO <sub>3</sub>	50.0
Biotin	0.05	NaH <sub>2</sub> PO <sub>4</sub>	200.0

These components were kept as ten separate stock solutions. The stock solutions were mixed as needed and adjusted to pH 7.2.

When the eye and antenna discs were cultivated with the brain hemispheres and the ventral ganglion, evagination was observed after 16 hours. Moreover, the eye discs extended along the dorsal surface of the brain, and continued to increase in area. After fixing in 10% formalin and staining in hematoxylin, the eye discs were found to possess the layer of the ommatidia. When the eye and antenna discs were cultivated without other organs, the difference in growth rate between the eye discs of the wild strain (Oregon) and those of the Bar strain was shown very remarkably. At the beginning of cultivation the ratio of the area of the antenna disc to that of the eye disc in Oregon was 1.00 to 1.22, whereas that in Bar was 1.00 to 0.91. After cultivation for 16 hours the ratio in Oregon became 1.60 to 2.37, that in Bar 1.89 to 1.57.

Kuroda, Y. Tissue culture  
of wing discs of D. melanogaster.

The wing discs of third-instar larvae were cultivated under sterile conditions by the same procedures described in the preceding note. The wing disc of the Oregon strain was shown to increase in area, and the median crossridge became clear. The peripheral concentric fold extended about the margin of the disc. The growth of the wing disc continued for about 24 hours. The wing pouch lengthened considerably. In the wing disc of the vestigial strain (vg), the center portion of the concentric fold was smaller than in that of Oregon. Even after cultivation for 16 hours the increase in area of the wing-forming portion of the vestigial strain did not equal that of the thoracic-forming portion.

Lederman-Klein, Ada.

Viability and fertility of a dachsous allele.

The  $ds^{52k}$  allele, on extraction from a wild population by the Cy L method had a viability of 49.9% of the expected. In further experiments at  $25^{\circ}\text{C}$  the viability

of the  $ds^{52k}$  homozygote varied from 7.3% to 56.9%, whereas heterozygotes of the type  $ds^{33k}\text{Pm}/ds^{52k}$  had viabilities from 68.8% to 103.9%. The superiority of the heterozygote cannot be ascribed solely to the presence of two different alleles at the  $ds$  locus, since the whole  $\text{Pm}$  complex might be involved. No significant deviation from the 1:1 sex ratio was observed in any of the experiments.

The time and mode of action of semilethal genes deserve the same attention as has been devoted to lethal factors (cf. Hadorn, 1951, *Adv. Genetics* 4: 53-83). Pronounced differential mortality during the embryonic, larval, and pupal stages under a chosen set of conditions could be excluded after the following experiment was conducted: Cy L/ $ds^{52k}$  flies were allowed to oviposit for two hours on a small cake of food, which was then transferred to a dish with abundant culture medium and kept at  $25^{\circ}\text{C}$ . One hundred or less prepupae were transferred to half-pint bottles containing much less than the normal amount of food. The counts made of adults shortly after eclosion are summarized in Table 1. The rate of eclosion of the  $ds^{52k}$  homozygote equals

Table 1

Parents	Cy L/ $ds^{52k}$		Cy L/+		
	Offspring	No.	%	No.	%
Prepupae	658			134	
% emerged		83.1			82.8
Adults: Cy L	402	73.5		82	73.9
non-Cy L	145	26.5		29	26.1

almost exactly that of the control homozygotes. Since in normal culture bottles there were always some  $ds$  flies drowned in the medium, the high rate of emergence in this experiment was ascribed to the dry atmosphere in the bottles with minimum amount of food.

The feeding capacity of  $ds$  flies was tested in tubes containing slants of food colored with a carmine suspension. After disappearance of the pupal fat body the red gut-contents could be observed through the abdomen wall. On dissection of etherized flies it was noted in both normal and mutant types that reversed peristalsis concentrates all the gut content rapidly in the crop. On dissection the fat body of the  $ds$  flies did not appear different from that of normal controls.

The effect of moisture and consistency of the culture medium on longevity of  $ds$  flies was examined by keeping newly eclosed adults in two sets of 10 test tubes, one of which received ordinary 'moist' food whereas the other was made up with slants of medium containing 2% instead of 1% agar. Seven pairs of flies were put into each tube. The results (Table 2) confirm the favorable effect of the dry slant on longevity of the  $ds$  homozygote, as well as the superiority of the  $ds^{33k}\text{Pm}/ds^{52k}$  heterozygote. Both types succumbed

Table 2

Type of flies	Average life span in days $\pm$ S.E.	
	Standard medium	"Dry" slants
Canton-S		
ds <sup>33k</sup> Pm/ds <sup>52k</sup>	> 9.396	> 9.769
ds <sup>52k</sup>	2.90 $\pm$ 0.124	4.04 $\pm$ 0.146
	2.34 $\pm$ 0.133	3.26 $\pm$ 0.190
Probability for differences between means (t test)		
ds <sup>33k</sup> Pm/ds <sup>52k</sup>		< 0.001
ds <sup>52k</sup>		< 0.001
ds <sup>52k</sup> versus ds <sup>33k</sup> Pm/ds <sup>52k</sup>	0.01-0.001	0.01-0.001

long before the average normal fly, in many cases because of sticking to the food with their spread wings or their short legs. Experiments carried out at different temperatures also indicated that humidity is a more important factor than heat, in determining viability.

Another deleterious effect of the ds gene is low fertility. When crossed with various strains, ds<sup>52k</sup> males yielded a few offspring. So did females, provided that their wings were cut off. Crosses of ds<sup>52k</sup> inter se were invariably sterile. In contradistinction to the findings of Stern and Bridges (Genetics 11: 503-530, 1927) for ds<sup>3</sup>, dissection of ds<sup>52k</sup> females showed that the ovaries were present, but that they averaged 10.8 ovarioles instead of 12.2 as found in Cy L/ds<sup>52k</sup> heterozygotes. The ovaries of ds<sup>52k</sup> flies were shorter and contained more than one row of mature eggs. Testes of ds<sup>52k</sup> males, when uncoiled, measured 2.225 mm average as against 2.750 mm average for Canton-S males; they were not coiled as regularly as those of the control flies, and showed various protruding bulges.

Lefevre, G., Jr., and  
Farnsworth, P. C. Further  
studies on reverse mutation.

have been inspected. The genetic constitution of the F<sub>1</sub> females was such that a reverse mutation of f occurring in the irradiated X chromosome can be clearly distinguished from a spontaneous reversal occurring in the unirradiated chromosome. Five complete reversals of f have been identified, as well as several partial reversals. All five of the complete reversals were fertile, but two of them arose in the unirradiated chromosome. The three presumably induced reversals occurred only after the exposure of f alleles (f<sup>1</sup> and f<sup>3N</sup>) shown previously by M. M. Green to be capable of spontaneous reverse mutation. The rate of complete reverse mutations in the present series of irradiations is clearly no higher than that exhibited spontaneously.

At the present time, four separate forked alleles have been tested for X-ray-induced reverse mutation following exposure to a dose of 5000 r. Nearly 210,000 F<sub>1</sub> females

All but two of the partial reversals are associated with male-lethality. In one case thoroughly tested, the male-lethality results from a gross chromosomal aberration which almost completely eliminates crossing over throughout the X chromosome. Cases like this may well represent suppression of the f

phenotype rather than reverse mutation.

In all the experiments spontaneous or induced reverse mutations at the vermilion locus could also be detected, but none was found. However, spontaneous reverse mutation of  $w^a$  could be detected in  $F_1$  males, and one reversion to  $w^+$  was found.

Lefevre, G., Jr. Genetic differentiation between phenotypically identical white alleles.

By use of a special mutant stock, it is possible to divide white-eyed mutants into two different categories. The stock used contains the delta-4S inversion with its customary markers:  $y$ ,  $Hw$ ,  $m^2$ , and  $g^4$ .

In addition, a white allele of spontaneous origin ( $w^{481}$ ) is also present. This  $w$  allele produces pure white eyes in males and homozygous females; and no gross aberration in the neighborhood of the  $w$  locus is detectable in salivary-chromosome preparations. However, heterozygous compounds of  $w^{481}$  with certain other phenotypically white  $w$  alleles result in the appearance of a slight mottling, which becomes more pronounced with age. Compounds with other, phenotypically identical,  $w$  alleles show either no evidence of mottling or else only the faintest trace of mottling, even in very old flies. Compounds with known deficiencies of the  $w^+$  locus remain pure white. In compounds with intermediate alleles such as  $w^e$  and  $w^a$ , mottling is not seen, but the background color is probably sufficiently dark in such cases that the faint mottling would be undetectable even if present.

In studies made so far, only  $w$  alleles of spontaneous origin show the mottling phenomenon, whereas none of the more numerous X-ray-induced  $w$  alleles tested (even though male-viable) have evidenced the mottling. Further tests are in progress.

Lindsley, D. L. On the position of the right break point of  $In(1)w^{m4}$  and  $In(1)rst^3$ .

In a previous issue of DIS (25) the construction of a chromosome specifically deficient for the nucleolus organizer was described. This chromosome is a combination of the left end of  $In(1)w^{m4}$  and the

right end of  $In(1)sc^{L8}$ , and was considered to be deficient for only the nucleolus on the basis of the published break points of the two inversions. In later crosses, however, it was discovered that flies of the composition  $In(1)w^{m4}$ ,  $sc^{L8}/bb$  are  $bb$  in phenotype and that the composition  $In(1)w^{m4}$ ,  $sc^{L8}/bb^+$  is lethal. These results suggested that the  $bb$  locus is not within the  $w^{m4}$  inversion, but to the right of the right break point. To test this point the combination  $In(1)sc^4$ ,  $w^{m4}$  was constructed and carried in combination with the left end of the X of  $T(1;4)w^{m5}$ .  $In(1)sc^4$ ,  $w^{m4}$ ;  $Dp(1;4)w^{m5}/bb$  or  $bb^+$  is  $bb^+$  in phenotype; since  $Dp(1;4)w^{m5}$  and the left end of  $In(1)sc^4$  are known not to carry a  $bb$  locus,  $bb^+$  must be carried in the centromere region of  $In(1)w^{m4}$ . It has been reported that the  $bb$  locus of  $In(1)rst^3$  is within the inverted segment and thus occupies a distal position in the chromosome. If this were true, the combination  $In(1)w^{m4}$ ,  $rst^3$  should be  $bb$ -deficient, but  $In(1)w^{m4}$ ,  $rst^3/bb$  or  $bb^+$  is  $bb^+$  in phenotype. From these observations it has been concluded that the  $bb$  locus is also proximally located in  $In(1)rst^3$ . This conclusion is difficult to reconcile with Gruneberg's recombination data involving  $In(1)rst^3$ , but I suspect that he may have had some bristle mutant other than  $bb$  in his crosses.

Lindsley, D. L. Reverse mutation at the loci of white and forked.

In the past several years 12 cases of reverse mutation at the forked locus and 3 cases of partial reversion of white have been observed and should probably be recorded. From a cross of  $In(1)sc^8$ , cv v f males to homozygous  $Y^S X \cdot Y^L$ ,  $Ins(1)EN$ ,  $dl-49$ , y v f car females (in pairs) 11 of 179,536 progeny were  $f^+$ ; all these reversions occurred in the y v f car chromosome. A reversion of this f allele ( $f^*$ ) has also been recovered from a female of the constitution  $In(1)sc^8$ ,  $y^{def}$  cv v f/  $In(1)dl-49$ , y v  $f^*$  car; in this case crossing over, at least of the non-sister variety, can probably be discounted as being involved in the reversion.

From crosses of y w attached-X females x  $In(1)sc^8$ , EN males two matroclinous females with pigmented eyes were recovered. These females resembled  $w^a/w$  in phenotype; progeny tests gave homozygosis for w and the intermediate allele of w with the expected frequency. From a mating of a y w attached-X female which had been irradiated with 4000 r to normal males, two matroclinous daughters with intermediately pigmented eyes were recovered; progeny tests of these females also gave homozygosis for white with the expected frequency.

Liuers, Th. Behavior of the nucleolar satellite in the neurons of *Drosophila*.

In 1949 Barr and his associates demonstrated a morphological sex difference in the nuclei of the neurons of mature cats. The difference depends on the size of the nucleolar satellite, which is well developed in females and very small or lacking in males. Later on the authors extended their observations to a great number of animals and other tissues. There was no sex difference in certain rodents. We have been interested in the nuclear morphology of the large nerve cells in the central nervous system of the adult *Drosophila*. We have studied 101 individuals of *D. melanogaster* and 21 of *D. funebris* in serial sections 5 microns thick stained with cresyl violet. In most animals of both sexes some of the neurons contain a well-developed nucleolar satellite, which is located immediately adjacent to the nucleolus. In 7 females and 6 males of *D.m.* no satellite could be found in any nerve cell. In 3 females and in 1 male of *D.f.* one of the neurons contained diplococcuslike satellites. In a small portion of animals of both sexes satellites of a threadlike type could be seen in some neurons. Barr postulates that the nucleolar satellite may be derived from the heterochromatin of the sex chromosomes. In females the satellite is formed by fusion of the heterochromatic parts of the two X chromosomes, in males of X and Y. In species having a small Y there would be a difference in the size of satellites in intermitotic body cells, according to sex. In *D.m.* and in *D.f.*--both species having a large Y--no difference could be detected between the sexes in the size of satellites. Exact measurements have not yet been made. Examination of specimens having different amounts of sex chromosomes is in progress.

Liuers, H. Test of Miracil for mutagenic activity in *Drosophila*.

Miracil D (BAYER), 1-diethylaminoethylamino-4-methylthioxanthone, has been found active in the therapy of Schistosomiasis. Among other effects, the egg and sperm production of the worms are disturbed within a few days. The substance also shows a slight inhibiting effect on some tumors of the rat. The compound was tested for mutagenic action by the Muller-5 technique. A solution of 0.025% Miracil, 2.5% acetone (to increase the solubility of the substance in water), and 5% sugar was fed to imagines by setting them for varying intervals on saturated glass filter plates, supplemented with a trace of yeast. In the applied concen-

tration the substance has no toxic effect and does not reduce fertility. The treated males were given fresh mates at three-day intervals. In 10-day treatments the males gave an average of  $0.55\% \pm 0.11\%$  lethals (24 lethals in 4,377 chromosomes tested) in the first four broods. Broods 5-13 gave an average of  $0.20\% \pm 0.10\%$  (4 lethals in 2,031 chromosomes tested). Controls treated in the same way with acetone, sugar, and yeast only, gave  $0.08\% \pm 0.05\%$  lethals (3 lethals in 3,925 chromosomes) in the first two broods. Treatment for three days only did not raise the mutability above the spontaneous level. In the offspring of four treated males, three clusters of two lethals and one cluster of three lethals were found in the later broods. The lethals of each cluster gave identical crossover values in genetic analysis. Five lethals of the clusters and one other lethal were associated with gross chromosome aberrations.

Lining, K. G. Double mutations at the white locus.

In the past year there have appeared two cases of  $F_1$  daughters from irradiated males with mutations to two separate  $w$  alleles.

In the first case a Canton S male was irradiated and mated to  $y\ w\ sn$  females. One daughter had one white eye and one partly colored eye. She was a gonad mosaic, as among the progeny there appeared some individuals with  $w$  eyes and others with slightly variegated red-brown eyes. They have not yet been further studied. In the second case a Muller-5 male ( $sc^{S1} B\ Ins\ w^a\ sc^8$ ) was irradiated and mated to  $y\ w\ sn$  females. One daughter had one white eye and the other eye partly white and partly red-brown, distinctly different from the original  $w^a$  color. This case gave only offspring with the colored  $w$  allele. Both cases will be studied further.

Lining, K. G. Effect of oxygen tension on chromosome breaks and recessive lethals.

Baker and Von Halle have recently shown that there is a considerable difference in the rates of dominant lethals induced in spermatozoa inseminated on the first and on the second day after irradiation in

air, but seemingly no difference when irradiation is carried out in nitrogen atmosphere. This phenomenon has been studied further in some experiments described below. Muller-5 males were irradiated in air or in nitrogen atmosphere when 0-1 or 3-4 days old, and were immediately mated to  $y\ w\ sn$  females for 24 hours and then transferred to new virgin  $y\ w\ sn$  females for another 48 hours. The impregnated females were allowed to lay eggs after the removal of the males. The  $F_1$  offspring was inspected, and individuals which were wholly or partly yellow (minute rearrangements) were noted as well as so-called hyperploid males (phenotypically gray among the normal  $y\ w\ sn$  males). Some of the  $F_1$  daughters were tested at random to determine whether recessive lethals had been induced. All results are presented in the table.

In the air series there were about 50% more yellow individuals and over 50% more hyperploid males in the A 1 than in the A 2-3 series, both when 0-1-day-old males and when 3-4-day-old males were irradiated. The contrast between the recessive lethals is much less, which agrees with the author's earlier finding that the rates of recessive lethals do not vary as much as the rates of chromosome breaks. Comparing the rates of yellow mutations and hyperploid males in the A 1 versus  $N_2$  1 and A 2-3 versus  $N_2$  2-3 series, it is evident that at low oxygen tension ( $N_2$ ) there was a lower rate of chromosome breaks than at higher oxygen tension (air). In the  $N_2$  1 and  $N_2$  2-3 series there was a large difference only if the males were irradiated within one day

after emergence. The cause of this effect of age at treatment will be further studied. The rates of recessive lethals in the N<sub>2</sub> series were much lower than those in the A series. For example a comparison of the results of irradiation of 0-1-day-old males of the A 2-3 series with those of the N<sub>2</sub> 1 series shows that the number of break-dependent chromosome aberrations was the same in both series but that there was a large difference in the rates of recessive lethals. This indicates that oxygen tension affects the occurrence not only of chromosome breaks but also of apparently break-independent, intragenic mutations.

Age of males at treatment (days)	Time of insemination after treatment in air or N <sub>2</sub>	Number F <sub>1</sub> daughters	y mut.		Partly y		Hyp. ♂	Recessive lethals		
			No.	Per 10 <sup>4</sup>	No.	Per 10 <sup>4</sup>		Abs n:r	Per 10 <sup>4</sup>	No. X chromosomes tested
0-1	A 1	43343	101	23.3	18	4.15	42	9.69	3327	7.45
	A 2-3	47216	81	17.5	9	1.91	25	5.29	3039	
0-1	N <sub>2</sub> 1	57978	105	18.1	16	2.76	29	5.00	2765	4.56
	N <sub>2</sub> 2-3	68078	58	8.51	11	1.62	25	3.67	2387	
3-4	A 1	34361	113	32.9	12	3.49	47	13.7	2634	8.92
	A 2-3	40657	87	21.4	5	1.23	30	7.38	2669	
3-4	N <sub>2</sub> 1	43463	68	15.7	11	2.53	19	4.37	2174	5.06
	N <sub>2</sub> 2-3	57363	78	13.6	11	1.92	31	5.40	2164	

Makino, S., Momma, E., and Wakahama, K. Diurnal activity of D. auraria.

Flies were collected by means of banana traps. Observations carried out in the shrubbery of the Botanical Garden, Sapporo, Japan, revealed that flies of this species were bimodal in their diurnal activity, both in cloudy and in bright weather. In the thick forest they proved to be bimodal in diurnal activity when the sun was shining, but not on cloudy days, regardless of similar conditions of light, temperature, humidity, and wind.

Martin, G. A., and Bell, A.E. Adult body weight in D. melanogaster as a trait for studies in quantitative genetics.

In studies of adult body weight of D. melanogaster, we are using a "Micro Gram-atic Balance" to get rapid and accurate individual weights. Under our cultural conditions, males average about 750 micrograms and females about 1100 micrograms, with coefficients of variation of 11.7 and 8.5 per cent, respectively. Of the 66 possible crosses from 12 inbred lines, 61 had males larger than the average of the parental males, and 65 produced females larger than the average of the parental females. The average superiority of all crosses was 40 and 169 micrograms, respectively. These preliminary studies are being expanded into a more detailed investigation.

Mather, W. B. The genus Crostophila in Queensland I.

The following is a summary of a paper prepared for the University of Queensland Papers, which will be published at an early date.

Ten species of the genus *Drosophila*, including six new species, are described and illustrated, as eggs, larvae, pupae, and adults: *D. cancellata* sp.n., *D. enigma* Malloch, *D. opaca* sp.n., *D. maculosa* sp.n., *D. levis* sp.n. *D. serrata* Malloch, *D. takahashii* Sturtevant, *D. dispar* sp.n., *D. versicolor* sp.n. These species have been assigned to subgenera and species groups. Their geographical distribution is recorded, together with the known Queensland distribution of *D. busckii* Coquillet, *D. melanogaster* Meigen, *D. simulans* Sturtevant, *D. ananassae* Doleschall, *D. hydei* Sturtevant, *D. repleta* Wollaston, *D. immigrans* Sturtevant, and *D. spinofemora* Patterson and Wheeler. These eighteen species are keyed. The taxonomy of the subgenus *Pholadoris* is discussed, and three new species groups are established, namely, *coracina*, *maculosa*, and *levis*. A new species group within the subgenus *Sophophora*--namely, *dispar*--is established.

Meyer, Helen U. Influence of maternal genotype on the hatchability of fertilized eggs in *D. melanogaster*.

parent females homozygous for the markers bw sp; ri e in chromosome 2 and 3 had been mated to homozygous al b cn sp males with wild-type chromosome 3. The reciprocal "cross 2" was made with al b cn sp females and bw sp; ri e males as parents. The proportion of eggs from which larvae emerged was much lower in cross 1 than in cross 2.

All eggs were known to be fertilized; they had already reached the polar cap stage when selected for further culturing, since this material was part of an experiment using the polar cap method for ultraviolet treatments. A similar and proportional difference in egg hatchability was observed in both UV-treated and untreated lots. Only data from untreated controls, consisting of dechorionated, fertilized eggs which had been observed to be in the polar cap state, are presented here.

Cross	No. eggs cultured	Proportions (%)		
		larvae/eggs	flies/larvae	males/tot.no.flies
(1)	1257	26.5	75.1	47.7
(2)	819	70.8	77.7	47.5

The table shows that only 26.5% of the embryos derived from cross 1 reached the larval stage, as compared to 70.8% of those from cross 2; this latter percentage is in agreement with our previous data for carefully handled dechorionated eggs. The next column lists the proportion of larvae which reached the mature fly stage, and shows very similar values for both origins, 75.1% and 77.7% respectively. This indicates that the larvae had an equal chance to complete their development once they were able to feed on the culture medium provided.

Since they were derived from reciprocal crosses, all embryos were of the same genotype except, in the case of the males, for the X and Y chromosomes. If the excess in embryonic mortality in offspring from cross 1 were caused by a sex-linked embryo-lethal, present in heterozygous condition and widely distributed in the stock furnishing the bw sp; ri e parent females--but not in

the *al b cn sp* stock--this also could account for the difference in hatchability. Then, the eggs which failed to develop would be male, and their death should be apparent in the sex ratio of the hatched adults. A comparison of the proportion of males among the total number of flies hatched, however, shows it to be similar in both crosses, 47.7% and 47.5%. This does not support the assumption of a sex-linked, widely distributed embryo-lethal in one of the stocks used.

It is therefore likely that the failure of so many embryos to progress from the polar cap to the larval stage in the case of cross 1 was due to abnormal or inadequate material supplied by the *bw sp; ri e* parent females, which generally are of lower vigor than the alternative type of females used in cross 2. It seems, then, that not only is the genetical constitution of the embryo itself of importance (as shown by the action of many lethal mutations) but also the genotype of the parental female plays an important part in the progress of early development.

(Work supported by grant from the American Cancer Society on recommendation of the Committee on Growth of the National Research Council.)

Mickey, George H. Analysis of mottled and Minute mutants.

Analysis of a large group of mottled mutants produced by radiations of various kinds revealed three categories: the white-mottled, the brown-variegated, and a miscellaneous group. Some are inversions, some translocations, and some more complicated rearrangements (e.g., one involving 4 breaks in three chromosomes), but all appear to be position effects related to the heterochromatin. Among the Minute effects, 60% were found to be in the second chromosome, 20% in the third chromosome, and about 13% in the fourth chromosome. One Minute proved to be a complicated transposition-inversion aberration involving six breaks in the two arms of the third chromosome.

Milani, R. Inheritance of *Musca domestica* mutants.

The mutant characters *ocra* (ochre eyes), *vti* (*vena transversa interrupta*), *gap* (section missing in *I4* vein), and *Kdr* (DDT knockdown resistance) of *Musca domestica* have proved to be individual recessive autosomal characters; no evidence of linkage has been found between *ocra* and *vti* or *gap*. *Kdr*, in the homozygous condition, gives full resistance to DDT (1 gr/m<sup>2</sup> on glass surface, 5 hours' exposure); it segregates very sharply both in inbred and in backcrossed *F<sub>2</sub>*'s; all the knockdown-resistant flies are also kill-resistant. The four mutants tested gave good and regular segregations in backcross tests; the same cannot be said for the *F<sub>2</sub>*'s because the data neither fit the expectation nor are they homogeneous. In some cases, lethals and inhibitory factors have been shown to be involved.

Milani, R., and Rivosecchi, L. Gynandromorphism and intersexuality in *M. domestica*.

Several gynandromorphs have been collected in wild populations or found among *F<sub>1</sub>* offspring of wild flies from the same places. Some laboratory stocks have endemic incidence of sexually abnormal specimens. A line has been carried for four generations with single-pair matings, and in each generation many such specimens have been found in variable proportions in several cultures. The distribution of heterosexual tissues is highly variable; however, specimens from the same culture are often very similar. The most extreme cases are males with female gonads and females

with small male chitinous parts on their sexual apparatus. In many cases it is difficult to decide whether a particular structure shows gynandromorphism or intersexuality; some specimens clearly show both types of abnormality in different parts of the body. The sexual behavior has not yet been studied, but some occasional observations have been recorded. The following seem of interest. Antero-males accept copulation and can lay fertile eggs. No interest in females has been observed (head male, behavior female). Antero-females attempt and can achieve copulation with females (head female, behavior male). Specimens with only small bits of male tissue on the sixth sternite have twice been observed mounting like males. Similar specimens have accepted copulation with males, giving normal offspring. On three occasions, complementary gynandromorphs reached copulation and produced batches of fertile eggs, which hatched normally; but no larvae reached the pupal stage.

Milani, R., and Rivosecchi, L.  
Sinistral coiling of male genitalia in M. domestica.

A recessive monogenic mutant has been found, which inverts the direction of coiling of male genitalia, which are thus coiled counterclockwise. It is fully penetrant, but the expressivity is not uniform. Some males have the hypopygium and terminalia perfectly developed, others show incomplete rotation of the hypopygium and abnormalities of synsternite 7-8. Two stocks of common origin differ in frequency of males with complete coiling. All males are sexually active, but not all can reach copulation because of mechanical difficulties.

Miller, D.D. The sex comb index as a basis for separating males of D. affinis and D. athabasca.

This ratio was determined for males of laboratory strains (kept at 19° C) of D. affinis and D. athabasca (100 males of each species, the strains being equally represented). The mean values and ranges for the different strains were as follows: D. affinis--Illinois 2.25-0.17 (2.00-2.50), Massachusetts 2.44-0.15 (2.10-2.78), Nebraska 2.11-0.19 (1.82-2.56), Tennessee 2.17-0.24 (1.67-2.50), and Texas 2.01-0.16 (1.70-2.40); D. athabasca--New Jersey 3.67-0.33 (3.00-4.50), and New York 3.57-0.30 (3.00-4.14). D. affinis generally had values of 2.5 or less (only 4 exceptions), whereas D. athabasca always had values of 3.0 or more.

The sex comb index was applied to 280 wild-caught males obtained at Lincoln, Nebraska, during 1952 and judged in advance (i.e., before knowledge of this criterion) to be D. affinis or a mixture of D. affinis and a few D. athabasca. These were found to have values of the index ranging from 1.60 to 2.56, and were concluded most probably to be D. affinis only. Collections in Michigan (Univ. Mich. Biol. Sta.) in July, 1954, yielded 12 males judged by inspection (but with the sex comb index in mind) to be D. affinis, and 150 similarly judged to be D. athabasca. Those classed as D. affinis were found to have sex comb index values ranging from 1.96 to 2.24, whereas those classed as D. athabasca were found to have such values ranging from 2.63 (two males with values less than 3.0) to 4.64. Collections in Wyoming (Jackson Hole Biol. Res. Sta.) in August, 1954, yielded 85 males classed as D. athabasca, and these were found to have sex comb index values ranging from 3.33 to 4.85.

As pointed out by Novitski (1946), it is likely that D. athabasca is distributed across the North American continent, though collections in the

middle part of the continent have not been sufficient to show this. Recently a laboratory stock has been established from banana bait exposed in August, 1954, at Minnewaukan, North Dakota, by Miss Marjorie Wanner of the University of Nebraska. Although only a few males have so far been obtained, inspection of these as to sex comb index makes it seem most likely that the stock is D. athabasca. Since the collection locality is roughly equidistant between previously known D. athabasca collection points in South Dakota and Wyoming on the one hand and Minnesota on the other (Novitski, 1946), this collection supports the idea that the species has a continuous distribution across the continent.

During microscopic observation of the prothoracic legs of D. affinis and D. athabasca it was observed that some individuals of both species may have the sex comb tooth of the second tarsal segment duplicated. This was found in some males of the Texas laboratory strain of D. affinis. It was also observed in one individual each of the Nebraska (1952) collection of D. affinis and the Michigan and Wyoming (1954) collections of D. athabasca. Previously the North American species of the D. affinis subgroup had only been known to have one sex comb tooth on the second tarsal segment, whereas the single European member of the subgroup, D. helvetica, was reported to have two or three teeth in this position (Burla, 1948).

Miller, R. H. Competition between D. melanogaster and D. hydei.

supply of fresh food (infrequent replacement of food dishes), melanogaster is unable to compete against hydei. In the presence of ample fresh food, melanogaster competes successfully and an equilibrium is set up, similar to that observed by Merrell in the case of melanogaster-funebris competitions.

Mislove, Rhoda F. A constant source of XXY (free-X) females.

dm sn ♂. The FM3 balancer is described in this DIS under "Melanogaster - New Mutants"; the other X chromosome of this female has a deficiency from 3C2 to 3C6, inclusive, and is therefore lethal in the male. Since all the regular male progeny are lethal and all the regular female progeny are completely sterile, only the exceptional progeny maintain the stock.

Monma, E. New karyotype found in the genus *Drosophila*.

characters are now being studied. Investigations of oogenesis, spermatogonia, and ganglion cells indicate that the diploid chromosome number is 12, consisting of five pairs of V-shaped chromosomes of various sizes in addition to a pair of extremely minute dot-like ones. The X and Y chromosomes, the two largest V-shaped elements, are similar in configuration. The primary spermatocyte metaphase consists of 6 bivalent chromosomes. The secondary spermatocytes possess 6 dyads consisting of four V-shaped elements, a dot-like one, and a large V-shaped X or Y.

Moriwaki, D., and Kitagawa, O. "Female-producing female" of D. bifasciata found in Japan.

In population cages, D. hydei shows a marked preference for old food. D. melanogaster, on the other hand, shows marked preference for fresh food. In the absence of an ample

supply of fresh food (infrequent replacement of food dishes), melanogaster is unable to compete against hydei. In the presence of ample fresh food, melanogaster competes successfully and an equilibrium is set up, similar to that observed by Merrell in the case of melanogaster-funebris competitions.

A special stock has been developed to serve as a constant source of XXY (free-X) females. It is designated as follows: In(1)w<sup>m4L</sup> N264-84R, y sn/FM3, y<sup>51d</sup> sc<sup>8</sup> dm B 1/Y ♀ and

In 1941 (DIS-14), Buzzati-Traverso first reported an extreme case of sex-ratio in D. bifasciata, describing that a fertilized

In 1941 (DIS-14), Buzzati-Traverso first reported an extreme case of sex-ratio in D. bifasciata, describing that a fertilized

female caught in nature near Pavia, Italy, produced only female offspring. Magni (1953), in Buzzati-Traverso's laboratory, published further investigations of similar females collected in Italy. In Japan, one female collected at Asakawa near Tokyo last winter (December, 1953), was found to produce only female offspring. Later another female was discovered, among wild flies collected at Kumotoriyama near Tokyo last summer (July, 1953), which could not produce males. The first strain has been maintained here at 19° C as the stock "Sex-Ratio-J" (SR-J). It is remarkable that such exceptional females should be found independently, in widely separated places, if their origin is the same. And if some genic factor causing their aberrant sex-ratio has long been concealed in the natural population, it might be supposed that feature must be associated with another, such as high fecundity.

Morpurgo, G. A new pod stock with high penetrance in D. melanogaster.

A new pod stock has been isolated in our Institute from a ClB stock. The pod line shows pleiotropic action on various organs of *Drosophila*. The most conspicuous effect is upon the legs: 1, 2, or 3 legs may be lacking or variously deformed. Generally it is the last pair that is affected. This type may sometimes comprise as much as 80%-90% of the total population. Exceptionally, flies with seven legs are recorded. One or both halteres are often absent. Wings may be absent, or atrophied, deformed, blistered, vesiculated, or in extreme cases completely inflated into a balloon. Very frequent, but present only in males, is the character "wings spread at right angles." The flies often show breakage or abnormalities of the tergites. Individuals simultaneously affected in all these organs are very frequent. Occasionally other mutant types are observed: hemithorax, bithorax. A fly with three aristae has also been found. Mortality of the eggs is high, between 40% and 50%. No attempt has been made to establish in what chromosome the factors that cause such aberrations are located. It is probable that the Y chromosome may be concerned, since males alone show the character "wings spread at right angles." None of these characters appears in the offspring of a pod fly crossed with a non-pod fly. Attempts to regain the pod characters have so far been unsuccessful.

Morpurgo, G., and Russi, M.  
A new modifier stock of vg mutant.

From the vg stock a new stock has been isolated, in which the recessive mutation vg acts as a semidominant. Flies of this stock are phenotypically indistinguishable from the wild type. When they are crossed with vg flies, there appear in all cases normal flies and flies with incomplete wings; if crosses are made between males and females that both have incomplete wings, the offspring will consist of normal, vg and flies with incomplete wings, but not in a simple Mendelian ratio. It is also possible to obtain flies with incomplete wings from the crosses vg x vg and normal x normal, provided that these flies are derived from the preceding cross. The defective part of the wings is generally in the medial region, or it may be a V-shaped fragment in the distal region. The phenotypic appearance is very variable, however. This effect is attributable to the action of one or many modifier genes, situated on the second chromosome.

Mossige, Jeanne C. Mating pattern of D. melanogaster males.

is desirable to use up all mature sperm as rapidly as they become available.

The mating habits of Canton-S males have been studied in order to work out a satisfactory mating scheme for reliable brood patterns in radiation experiments, where it

All the experiments have been performed with Canton-S males and females at 25°. Individual males were mated successively to a certain number of females for 24-hour periods; the females were then isolated individually in vials, and each vial in which larvae were observed was recorded as a fertile mating. Egg counts were made in some experiments and some of the fertilized females laid large numbers of unhatched eggs, but all matings which gave any offspring were recorded as fertile. All females used were 2-3 days old and the vials in which they had been stored were examined for larvae as a check on virginity.

It has been found that newly eclosed males which have been isolated for 48 hours will fertilize up to 10 females during the next 24 hours. Average number of females fertilized by such males was:

1st day	2nd day	3rd day	4th day	5th day	6th day
8.5	6.5	4	1	2.3	1.5

and thereafter about one female per day as long as the males lived (about 20 days).

When 0-4 hr old males were mated individually with 5 females every 24 hours the average number of females fertilized per day was 2.9, 3.8, 2.6, 3.5, 2.2, 2.3 and 2.8 for the 1st to 7th days respectively.

In another experiment three groups of 10 males were mated individually as follows:

	Ave. no. ♀♀ fertilized on 4th day
Group I x 1♀ for 3 days	6.8
Group II x 1♀ per day for 3 days	6.5
Group III x 5♀ per day for 3 days (2, 2.5 and 4)	4
Groups I, II, and III x 10 ♀♀ on 4th day.	

This experiment indicates that males do not mate as readily with fertilized females as with virgins so that leaving them with the same females does not afford the males a maximum opportunity to copulate. To test this further a large number of virgin Canton-S females were mass mated with M5 males for two days, then the females were isolated individually in vials for two days to test fertilization and 30 fertilized females were mated in groups of 10 to single 0-24 hour old + males. The females were again isolated individually in vials after 24 hours, and only one of the 30 females, after examination of all of the progeny, proved to have been fertilized by a + male, thus confirming that the males in these experiments do not mate readily with fertilized females.

Finally a test was made with newly eclosed Canton-S males irradiated with 2500 r and mated immediately to 5 females, and transferred to 5 new females each day. These males fertilized an average of 2.6, 3.6, 4.6, 3.7, 3.0, 2.8, and 1.4 females on the 1st to 7th days respectively. On the 8th and 9th days only one female was fertilized in 10 groups of 5♀ x 1♂ and from the 10th to the 20th day each male fertilized on the average slightly more than one female per day.

It seems necessary, therefore, to mate males with at least 5 females each day during the first week, and thereafter with at least 3 females per day in order to obtain clearcut brood patterns to study sensitive stages. The drop to almost 0 on the 8th and 9th days in the X-ray experiment would seem to indicate that certain stages can be fairly well isolated and that this mating scheme affords the possibility of producing broods with little overlapping from sperm which have been irradiated during the same stage of development.

The question of the influence of mating frequency on sperm production cannot be answered conclusively by these experiments but if the total fertile matings for the first 6 days after eclosion are compared we find 20 for the males isolated for 2 days and mated successively on the next 4, and 17.3 for the males mated each of the first 6 days, a small difference and in a negative direction. Further experiments are in progress.

(Work supported by a grant from The Norwegian Research Council for Science and the Humanities and carried out at Norsk Hydro's Institute for Cancer Research, The Norwegian Radium Hospital, Oslo.)

Muller, H. J. A semi-automatic breeding system ("Maxy") for finding sex-linked mutations at specific "visible" loci.

In order to facilitate the making of crosses designed for the detection of mutations at specific loci, two stocks, designated for short as "Maxy" and "Maxy-v," have been constructed. The females of both stocks carry one multiple-mutant X chromosome of composition  $y\ ac\ pn\ w\ rb\ cm\ ct^6\ sn^3\ ras^2\ v\ dy\ g^2\ f\ Tu\ car$ , and another X chromosome with the normal alleles of these mutant genes. In the latter chromosome a mutation to any of the above recessives (i.e., to all but *Tu*) would be recognizable, in view of the fact that all of them are already present in the multiple-mutant chromosome. To minimize crossing over, this other chromosome is provided with two moderate-sized inversions, those of *In49* and *B<sup>M</sup>1*, and for purposes of balancing it is provided with the lethal, *lJ1*, present in association with *scute-J1*.

The males of these stocks are provided with a Y designated as *lJ1<sup>+</sup>.Y*, which arose by a neutron-induced minute deletion of *y<sup>+</sup>* and *ac<sup>+</sup>* from the *sc<sup>8</sup>* region of a *sc<sup>8</sup>.Y*, and which still covers *lJ1* and therefore allows males with the *lJ1 scJ1 In49 B<sup>M</sup>1* chromosome to live and breed. They show the *scJ1* and *B<sup>M</sup>1* phenotypes, but any newly arisen mutations at the fourteen specific loci in question would be recognizable in them. On breeding with their heterozygous sisters they produce viable daughters which are practically all (except for rare mutants and crossovers) like their mothers, since the homozygous *lJ1* females die; hence virtually all daughters and sons are useful for the detection of mutations at the loci in question, and it can be known whether the mutation arose in the paternal or maternal X chromosome. A few multiple-recessive males do arise, but not enough to be troublesome for the detection of mutations or for the breeding scheme. Occasionally, viable crossovers occur, derived from exchanges to the left of *In49*, giving phenotypically *y ac* or *y ac pn* or *y ac w dd* or *QQ*, or between *In49* and *InB<sup>M</sup>1*, giving *y ac w ct sn dd* or *QQ*. None of these are confusable with mutants. By choosing females showing heterozygous *Tu* for breeding one can also avoid getting *B<sup>M</sup>1* by crossing over (between *In49* and *B<sup>M</sup>1*) in place of *f Tu car* in the multiple-mutant chromosome.

The Maxy-v stock is just like the Maxy one except for the presence of vermillion (v) in its *lJ1 scJ1 In49 B<sup>M</sup>1* chromosome, which causes both males and females of this stock to show the v color. An experimental series is started by the cross of Maxy-v virgin females to Maxy males, or the reciprocal cross, the purpose of the crossing being to allow the recognition and discarding of nondisjunctionally produced offspring and of their sibs, in cultures yielding such offspring (since the presence of an extra Y upsets several features of the scheme). In general, breeding may then be continued for generation after generation, selecting in every alternate generation v *Tu* (otherwise normal) *QQ* and *Tu dd* and in the other generations *Tu QQ* and v *Tu dd*, from the cultures not showing nondisjunction. (Females showing *scute-J1* are also indicative of the presence of an extra Y, whose *lJ1<sup>+</sup>* portion made these homozygotes possible.) For work on

spontaneous mutation, or mutagenesis by chemical treatment of the food, it is unnecessary to obtain virgins when using Maxy, unless one wishes to control the aging of the sperm or the time of insemination in some exact way.

During the past year, Mr. F. Verderosa has examined slightly more than 120,000 flies ( $\text{♀♀} + \text{♂♂}$ ) for spontaneous mutations, in a series of the alternating Maxy x Maxy-v crosses in which most of the sperm had been aged in the female for about 2 weeks. Only two cases of actual mutation in one of the loci in question (unfortunately discarded before analysis) were found among them: one of a viable cut which appeared in two male progeny because of its having arisen in a premeiotic female germ cell; and one of a garnet-like eye color, which appeared in a female and hence presumably arose in a male germ cell. Thus the spontaneous mutation rate in the stock appears to be low, as compared with earlier results of Muller, Valencia, and Valencia (1949) on spontaneous mutations arising in the female, using the "plond" stock. The present stocks also lend themselves readily to the detection of lethals arising in the paternal LJ1 sc<sup>J1</sup> In49 (v) B<sup>M1</sup> chromosome, by breeding the females in individual cultures.

Several stocks similar in principle to Maxy were made up before it was. The first was called "Max" (see our stock list) to denote the fact that it was designed for the finding of mutations in the males' X's, but in this and the other earlier stocks it was not possible to detect the origination of yellow. Because of its incorporation of the arrangement for detecting yellow, the present stock has therefore been designated "Maxy."

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Muller, H. J. A stably breeding attached-X stock ("sncc") designed for discriminating between deletional and other "detachments."

Inspection of the phenotype, whether or not the X-chromosome found has been provided at its broken end, on the "stump" to the right of the centromere, with a "cap" derived from the distal end of that homologous chromatid (or its sister) to which the stump had belonged. Such a capping would constitute, in effect, a large deletion. This purpose was achieved by Rapoport (1940, Doklady 29: 612-615) by constructing attached X's one member of which was provided with  $y^2$  (with dark bristles) and the other member with  $y$   $Hw$ , and by providing the latter member with the inversion derived from C1B, to hinder the production of cross-over daughters and thus perpetuate the heterozygosity. Rapoport also arranged (by an unstated method) to have no Y in these females, so as to prevent the X's from separating by crossing over with a Y.

We have taken the essentials of Rapoport's scheme and modified the details, with the aid of stocks now available, so as to make it somewhat better adapted for its purpose. On our scheme one member of the attached X's is provided with  $y$  and the other member with  $sc$ , but no lethal is present, so that either member, when separated, is viable in the male. In such sons, and also in daughters whose father's X contained  $y$   $sc$ , the first member when separated gives the phenotype  $y$  and the second member the phenotype  $sc$ , unless the broken stump, beyond the centromere, has been capped by a distal end derived from this homologous member (or from the latter's sister), in which case the phenotype is  $y^+$   $sc^+$ . In fact, where the member found is that having  $sc$  at its distal end, even

In the attack on the problem of whether a pre-formed telomere is required for *Drosophila* chromosomes or whether, contrariwise, healing of broken ends occurs, it is useful to have attached-X's with their distal ends differently marked, in order that, when separation of the X's occurs, it can readily be ascertained, by

capping by its own sister (nonhomologous) chromatid is recognizable, since the additional dose of sc results in a phenotype much more nearly normal, though not quite normal, with respect to this character, than the ordinary scute is. To these conclusions it is of course necessary to add the qualification that capping by the distal end of an X would not be recognizable by these means in those cases in which a y-containing member had been capped by a sister chromatid or in the rare cases in which the cap was so small as not to include the "covering" locus in question.

The heterozygous inversion used on our scheme is In49, which is present in the y-containing member. In order both to differentiate the two members otherwise than by their tips (since these may cover each other, and occasionally though very rarely may become homozygous by crossing over to the left of In49), and also to have a "check" (in both senses) on the occurrence of crossovers to the right of In49 which would cause homozygosity, the two members were provided within the region of the inversion with different recessive genes having visible manifestations and causing female sterility when homozygous, oc (along with  $ct^n$  and ptg) being provided for the member having sc, and  $sn^{X2}$  for the member having y. Thus all "diagonal" crossovers (i.e., those between one chromatid and the homologous chromatid which is sister to the strand to which the first is attached), giving X's homozygous for the region of the inversion, are automatically sterilized and eliminated from the stock. Occasional watch must be kept, however, to eliminate the very rare crossovers to the left of In49, visible by inspection, in which the X's have become homozygous for y or for sc. It is also to be observed that, by crossing over to the left of In49 between homologous attached chromatids, y and sc on very rare occasions will exchange places, with maintenance of the normal phenotype, and that these rearrangements will gradually accumulate in the stock. Though their products, on detachment, are recognizable, they make it advisable occasionally to remultiply the stock from individual females, with detachment tests of the composition of the resulting lines. A similar situation holds with regard to the gene car, which was introduced into the member with sc  $ct^n$  oc, since this too may exchange positions with car<sup>+</sup> by crossing over, to the right of In49, or may become replaced by homozygous car<sup>+</sup>; this gene, however, is not essential for the scheme. Neither is ptg, which accompanies oc in the formula only because it was already with it and is too close to it to be removed readily. Summing up the composition of the females of snoc stock, it may be represented as follows:

(no Y)	sc $ct^n$ oc ptg car
	y In49 $sn^{X2}$

The males of the snoc stock, in order that no free Y may be introduced, have been provided with Lindsley and Novitski's (1950, DIS-24) "X.Y" chromosome (having InEN and the y<sup>+</sup>-containing sc<sup>8</sup> attachment to the Y<sup>L</sup>), into which have been introduced the genes  $sn^5$  and oc (along with ptg, as usual). The function of  $sn^5$  and oc in the males' X's is to cause the automatic sterilization, within the stock, of females resulting from any form of separation of their mother's X's, since such females would be sterile compounds of  $sn^{X2}$  with  $sn^5$ , or else would be sterile by reason of being homozygous for oc. It is from these genes that the abbreviation "snoc," for this stock, is derived. At the same time, males arising from separation of their mother's X's would also be sterile, their sterility being caused by their lack of a Y chromosome. This still leaves as a possible cause of breakdown of the stock the formation of triploid females, but even in this case there would be very little chance for anything but reversion to the original arrangement, since InEN would be very obstructive to the occurrence of crossovers between the chromosome con-

taining it and one of the attached X's. Moreover, the results of such crossing over would soon be evident in the phenotypes of the male descendants.

When the stock is used for the study of detachment it serves only to provide the needed females, which are outcrossed, preferably (for reasons already explained), to males having some "strong" yellow and scute alleles, and provided with B, a long inversion, and a marker near the middle. Useful for this purpose is our stock having  $y\ sc^{Sl}\ B\ In49\ v$  males. The detection of the "detachments" and the discrimination between cappings by the X itself, of the types above mentioned, and other forms of separation, are then readily made in  $F_1$  flies of either sex, by observation of their phenotypes.

(Work supported by grant from the Atomic Energy Commission, Contract AT(11-1)-195.)

Muller, H. J. A stock for automatic accumulation of lethals arising in the female.

Where mutation frequencies are low, the study of them is often facilitated by methods for accumulating recessive mutations automatically over the course of several or many generations (n). Here the analysis can be postponed until

the last generation, which, provided the tests are made on individuals all derived from separate lines of descent, shows a frequency n times that of a single generation. (For strict accuracy we must modify the observed frequency by a factor representing such selection as has on the average operated against the heterozygotes because of the incompleteness of recessivity.) Schemes of this sort involving autosomes were put into operation as early as 1918 (Muller, 1928, Genetics 13: 279-357) but they have not yet been satisfactorily modified in such a way as to distinguish between mutations arising in the male and in the female. Moreover, tests of autosomes involve the additional labor of breeding to one more generation than is necessary with the X.

On the other hand, the sex chromosomes lend themselves to the construction of stocks for accumulation of recessive mutant genes in the female. The stocks called "plond" (DIS-22), "plynd" (DIS-23), and "jynd" (DIS-24) are of this kind, being designed for the accumulation, and for the finding in any generation, both of visible mutations at specific loci and of lethals. (The letters "nd" here denote nondisjunctional reproduction.) Only one of the X chromosomes of the female serves for this purpose in these stocks, however, the other X being provided with so many (some dozen) mutant genes for the recognition of allelic visibles in the first X that it is unsuitable for the finding of lethals in itself. We have therefore made up a stock which is similar in general construction but from which the recessive markers have as far as practicable been omitted, so that lethals can be detected in both X's of the female, although of course this stock is no longer adapted for the detection, by mere inspection of the females, of such visible mutations as may have arisen.

This stock, designated "facl" for short (to suggest the words "female accumulation of lethals"), has its females provided with two X chromosomes isolated by inversions from crossing over with one another and by the same means rendered prone, in the presence of a Y, to undergo nondisjunction and so to result in daughters receiving both X's from their mother, with resultant accumulation of mutations in the female line in both X's. To insure automatic perpetuation of this type of transmission, genes are provided which render both types of "regular" (disjunctionally produced) females sterile. Similarly, among the males, only the nondisjunctionally produced ones are fertile, since only they have the fertility genes of  $Y^S$  (which is attached to their X). Thus the original cross is repeated indefinitely, without selection by the operator.

In the form of the "facl" stock in our current list, the fertile females contain  $Y^{LC}$  (the ring-shaped Y deficient in a part at least of the set of  $Y^S$  fertility genes) and the following X chromosomes:  $y^2$   $oc$   $ptg$   $B^M1$  and  $sc^S1$   $fu$   $In49$   $sc^8$ . The fertile males of this stock are of the composition  $Y^{LC}/y^2$   $oc$   $ptg$   $fu.Y^S$ . Since these males carry both  $oc$  and  $fu$ , for which the females are heterozygous, one portion of the "regular" daughters is sterilized by being homozygous for  $oc$ , and the rest of them by being homozygous for  $fu$ , while, as noted earlier, the "regular" males are rendered sterile by their deficiency in  $Y^S$ . The secondary nondisjunction caused by  $Y^{LC}$  in the presence of the given heterozygous inversions is of a very high frequency, so that cultures of individual females are readily perpetuated. At the same time, such cultures do give enough "regular" offspring, and the viability of both types of "regular" males is initially high enough, to serve readily for the detection of recessive lethals, as well as of visibles, in both maternal chromosomes, when the males derived from individual females are inspected. Of course the very lack of selection against lethals and detrimentals in the maternal chromosomes makes it necessary occasionally to practice artificial selection of those cultures derived from individual mothers which give a satisfactory production of both types of regular males, and this is especially called for prior to the start of an accumulation experiment.

By using another female-sterile gene in place of  $fu$ , other forms of "facl" may be made up. Probably the best of those readily produced employs singed. The "facl-sn" stock is made up by crossing ordinary facl females to males of our stock  $sc^S1$   $In49$   $sn^X2$   $sc^8$  and then crossing several (about 30) of the daughters individually (to get one of the right combination) with males of our stock of  $Y^{LC}/y^w$   $sn^5$   $oc.Y^S$ . The singed-containing maternal chromosome usually has a better viability than that containing  $fu$ ; that containing  $oc$ , however, usually produces many regular male imagines.

Lethals and visibles arising in facl stocks serve, without the need for virginity, for the immediate establishment of balanced stocks of those mutants themselves, by merely crossing the females containing them to their fertile brothers.

(Work supported by grant from the American Cancer Society on recommendation of the Committee on Growth of the National Research Council.)

Muller, H. J. Multipurpose stocks for studies of mutagenesis.

It is often desirable to study the production of lethals, translocations, and losses of chromosomes and chromosome parts in the same individuals. This can be accomplished by the use of a pair of multipurpose stocks, one providing the primordial male parents ( $P_1 \delta\delta'$ ) and the other the primordial female parents ( $P_1 \varOmega\varOmega'$ ) of the given multipurpose mutation experiment. For work currently being carried out in our laboratory we have constructed a pair of relatively simple multipurpose stocks, designated for short as "multi- $\delta$ " and "multi- $\varOmega$ ." Their composition is as follows.

"Multi- $\delta$ ":  $sc^8.Y/y$   $In49$   $B$ ;  $bw^D$ . Here the  $sc^8.Y$ , present only in the males, covers the  $y$  of the  $X$ . The females are homozygous for the  $X$  indicated, and  $bw^D$  is homozygous throughout.

"Multi- $\varOmega$ ":  $X.Y$   $In47$   $y$ ;  $st$ . This stock contains the  $X$  chromosome--having  $Y^S$  at its left end and  $Y^L$  at its right end, with inversion of the whole euchromatic region with respect to the centromere--constructed by Lindsley and Novitski (1950, DIS-24 et seq.). It does not have the scute-8 attachment, and

the color of the flies is therefore yellow. No free Y is present. The stock is homozygous for *st*.

When males of the multi-♂ stock are crossed with females of the multi-♀ stock, the "regular" male offspring ( $F_1$ ) are gary ( $y^+$ ) brown, whereas those which failed to receive the paternal X or Y or which received an X with a large deletion or a Y from which the  $y^+$  had been lost or inactivated are yellow brown. Because of having an X.Y, however, they are usually fertile and their type of aberration can therefore be subjected to further genetic tests. At the same time, offspring produced by loss or nondisjunction of the maternal X's are likewise recognizable, as yellow Bar brown males (sterile) or gray non-Bar brown females. Results of nonvirginity of the mothers are also evident on inspection, as flies entirely like those of the multi-♀ stock (*y st*).

For the detection of sex-linked lethals, the  $F_1$  females need not be virgin but may be crossed to their brothers, or to males of any non-Bar stock containing a  $sc^8.Y$ , a long inversion (preferably scute-type or EN), but no In49. However, as noted below, it is helpful to have these males contain non-yellow and a conspicuous marker such as white. (The  $sc^8.Y$  is desirable, to guard against counting pre-existing bobbed-lethals or lethals in the  $sc^8$  region as being newly arisen, a danger demonstrated in as yet unpublished work of Abrahamson and Telfer.) Absence of Bar males in  $F_2$  indicates the presence of a lethal in the paternal X, absence of non-Bar males a lethal in the maternal X. In the former case, the  $F_2$  Bar females can be tested en masse, in matings with their brothers, to verify the lethal. A maternal lethal can be readily verified only if  $F_2$  females have been obtained from an outcross, as with  $sc^8.Y/w \delta\delta$ . The  $F_2$  females from the outcross are then distinguishable (from the  $F_2$  females derived from the cross with the  $F_1$  males) by being gray, and the lethal in the X.Y *y* chromosome will be evident by the fact that all (except nondisjunctionally produced *y B*)  $F_3$  males from these females (which may be bred en masse from any given  $F_1$ - $F_2$  culture) will be white.

For the detection of translocations, the  $F_1$  males may be crossed individually to virgin females like their mothers, that is, derived from the multi-♀ stock. The absence of one or both classes of recombinants involving any of the three pairs of markers (*y* vs.  $y^+$ ,  $bw^D$  vs.  $bw^+$ , and *st* vs.  $st^+$ ) considered two pairs at a time then indicates the presence of a translocation between the chromosomes with those markers or their alleles. Verification of a translocation involving the paternal chromosomes is obtained by en masse crossing of  $F_2$  males which are nonrecombinant in the respects in question by virgin females like the mothers (multi-♀). Verification of a translocation involving the maternal chromosomes, however, requires crosses of a more special kind. If it is anticipated that this type of testing will be desired, the females to which the  $F_1$   $\delta\delta$  were crossed may have been provided with *bw* and *e* in addition to the composition already designated for them. This makes possible the crossing of the  $F_2$   $\delta\delta$  in which a maternally derived autosomal translocation is suspected (these  $\delta\delta$  will be *st* in phenotype) to virgin *bw e* ♀♀ (or to virgin females like their mothers), for the detection of *bw e* and *bw*  $e^+$  recombinants among the  $F_3$ .

It is to be noted that the above arrangement allows the detection of three or more times as many translocations of the Y as are usually found, since the two thirds which are ordinarily sterile are here rendered fertile by the presence of the *y<sup>S</sup>* and *y<sup>L</sup>* on the X. Moreover, the  $sc^8.Y$ , having in connection with  $sc^8$  some heterochromatin derived from an X, is more subject to translocation than is an ordinary Y. To detect these translocations *y* vs.  $y^+$  is a better marker than sex, because of the frequency with which translocated Y's undergo nondisjunction with X's. In fact, the latter tendency should even allow many of the Y-IV translocations to be picked up on this system of

crossing, despite lack of a marker in IV.

For the detection of translocations between chromosomes II and III the present set-up presents the special advantage that most of these (all except those having viable aneuploids of  $bw^+$   $st^+$ , i.e. red eyed, type) can be detected by a routine observer looking with a hand lens at the unetherized flies within the culture vial to "spot" the presence of the very light eyed  $bw^D$   $st$  combinations. The presence of these shows that (with the qualification mentioned above) no large translocation of II with III has arisen in the given vial.

(Work supported by grant from the American Cancer Society on recommendation of the Committee on Growth of the National Research Council.)

Muller, H. J. Origination of a viable achaete deficiency by nearly homologous nonreciprocal exchange.

This deficiency includes the locus of achaete and possibly also that of yellow, but no other loci whose absence is phenotypically evident. It arose not by a minute deletion but by breakage in nearby positions in two homologous (non-sister) chromatids of an X-rayed oöcyte, the breakage in one chromatid being just to the right of the locus of achaete, between it and the locus of scute, and that in the other chromatid either just to the left of the locus of achaete, between it and yellow, or just to the left of yellow. The distal piece of the chromatid which had broken farther to the left then joined onto the proximal piece of the chromatid which had broken farther to the right, to reconstitute an X chromosome with the genes in their normal order except for the absence of the locus of achaete and perhaps also that of yellow. Thus the occurrence bore a superficial resemblance to "unequal crossing over." The reason it is not known whether the locus of yellow was lost is that the mutant gene for yellow was itself present in the chromatid which furnished the distal piece, and its phenotypic expression is not distinguishable from that of a deficiency for this locus (Muller, 1935, *J. Hered.* 26: 469-478).

The case was found by Herskowitz, as one of the cases of production of a "detached X chromosome" on irradiation of females having attached X's of ordinary type and no Y, in experiments to test the possibility of "healing" of broken ends (see Herskowitz and Muller, 1953, *Genetics* 38: 669 et seq.). The mother's attached X's had markers in the arrangement called "snoc," one of the X's having the composition  $sc\ ct^h\ oc\ ptg\ car$  and the other  $y\ In49\ sn^x2$ , the father being B. The exceptional offspring was a female of heterozygous B type. Further study, carried out by Muller, showed that sons of the exceptional female which received the whole "detached X" from her, without crossing over, were phenotypically  $sc\ ct^h\ oc\ ptg\ car$ , as though there had been healing of the break. However, crossing over occurred freely between the "detached X" and that containing B. Surprisingly, the crossover daughters which had received the left end of the "detached" X and the right end of the B-containing X, and which had received the  $y\ sc$  X derived from the male with which the exception had been crossed, were simultaneously yellow and scute-1 in phenotype, whereas those with both left and right end from the "detached" X were non-yellow but scute 1. This indicated (unless one assumed mutation to y) that the left end of the "detached" X had been derived from the  $y\ In49\ sn^x2$  chromosome by a break to the left of the scute locus, since scute-1 was not covered, but to the right of all phenotypically evident genes that are distal to y, since the corresponding male crossovers were viable and (except as mentioned below) normal. However, the rest of the detached X up to the centromere was obviously from the homologous  $sc\ ct^h\ oc\ ptg\ car$  chromosome,

which must have been broken between  $y^+$  and  $sc$ . At the same time (and substantiating the interpretation that the arm of the X which had furnished the major portion of the "detached" X had been broken between the loci of  $y$  and  $sc$ ), the right end of the exceptional chromosome must have received a small "cap" containing  $y^+$  but not  $sc$  (since an extra dose of  $scl$  gives a phenotype more normal than that of  $scute-1$ ). This cap was derived from the left end of an arm of  $sc$   $ct^n$   $oc$   $ptg$  car type, presumably from the same chromatid as that which had furnished the major piece.

Since according to this interpretation both the homologous chromatids which had participated in the formation of the "detached" X had broken within the very limited region bounded by the genes left of yellow on the left and by scute on the right, it was of special interest to determine whether these two breaks had been in identical positions, as they are at normal crossing over. The hatching (somewhat delayed) of the yellow Bar males having the "detached" X without its cap was therefore eagerly awaited, to determine whether or not the achaete character was expressed in them. It was in fact expressed to an extreme degree, as in the long-known ( $y ac^-$ ) deficiency caused by crossing over between the  $scute-8$  and  $yellow-3P$  inversions (Muller, 1935, *ibid.*). Thus the genetic evidence was rendered convincing that in the present case there is a physical deficiency of one or two genes, caused by breaks in homologous chromosomes.

Since the two breaks were in such nearly (though not quite) corresponding positions it appears likely that they were somehow associated in their origination, most probably by having both been caused by activations of the same cluster in one ionization track, whereas the third break, near the centromere, arose independently of them. That the two nearby breaks in homologous chromatids were not of the nature of induced crossovers, however, is indicated not only by the fact that the positions of breakage were slightly different, but also by the nonreciprocal nature of the connections formed. For although the left-hand piece of one chromatid (that of composition  $y$   $In49$   $sn^{X2}$ ) did join with the almost complementary right-hand piece of the "second," nevertheless the left-hand piece of the second chromatid, instead of joining with the almost complementary piece of the first one, joined with the stump produced by the proximal breakage, in the heterochromatic region of the first, the unions being of the "cyclic" or "rotational" nature.

Further genetic analysis showed that the cap of this detached X sufficed to cover deficiencies extending from the terminus up to and including  $ac$  but not beyond. The stock having this deficiency without the cap is designated  $y$   $ac^-$   $sc$ . As explained above, however, its composition may in fact be  $y^- ac^- sc$ , or, more briefly, ( $y ac^-$ )  $sc$ . It is noticeable that the bristles of the scute series are somewhat oftener absent than in ordinary  $scute-1$ , in agreement with earlier inferences that the fields of action of the  $ac$  and  $sc$  loci, because of the common origin of these genes from one another by duplication (Muller, 1935, *ibid* and *Genetica* 17: 237-252), still overlap to some degree.

It is believed that this is the first case in which it has been possible to show by genetic evidence that there is an *in situ* deficiency (i.e., one in which the chromosome structure is otherwise unaltered in the vicinity) of not more than two genes.

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Nawa, S., and Taira, T. The relation between eye pigment and pterins in D. melanogaster.

The pterins found in D. melanogaster were investigated. The relative amount of isoxanthopterin contained in the whole body of the males of various mutants of D. melanogaster.

separated by paper chromatography was measured by the fluorometric accessory set of a Beckman spectrophotometer. Males aged 15 days or older were used as material. It was confirmed by the agreement of Rf values, fluorescence, ultraviolet absorption spectra, and chemical natures that the fluorescent substances obtained from the material consist mainly of 2-amino-4, 7-dihydroxypteridine (isoxanthopterin) and derivatives of 2-amino-4-hydroxyppteridine. The relative amounts of isoxanthopterin in these mutants are as follows: v, 54; cn, 48; wild type, 43; pr, 43; dke, 35; st, 35; car, 35; cl, 32; ca, 27; pP se, 17; cm, 12; w<sup>a</sup>, 4; rb, 4; bw, w and v bw, 0.

It has been found also that the yellow eye pigment in the se and cl mutants is a 2-amino-4-hydroxyppteridine derivative and identical with xanthopterin-B found in the mutant "lemon" of the silkworm. Strains of D. melanogaster without any red eye pigment, like bw, w, and its alleles, have no pteridine compound, whereas strains having red or yellow eye pigment, such as wild type, cn, v, se, cl, etcetera, contain pteridine derivatives in large quantities.

These data suggest that there is a close relation between red or yellow eye pigment and pterins. Further work along these lines is in progress.

Nef, W. Speed of development and manifestation of the mutant Pearl (Pl) of D. melanogaster.

The time of development is much increased at low temperatures or in a low yeast concentration. If flies which are cultured in a certain low yeast concentration at

25° C have the same duration of larval and pupal development as those reared in normal food at 22° C, they show different degrees of manifestation. Flies grown in deficient food have more and stronger defects than others. Therefore it can be concluded that speed of development is not the only factor responsible for degree of manifestation.

Nicoletti, Benedetto. Some observations on the probable differential rate of growth among different genotypes of D. melanogaster.

With the purpose of investigating the possibility of a differential rate of growth among flies of different genotypes, a certain number of experiments with different mutants of D. melanogaster were performed. Some evidence in favor of the

existence of such a phenomenon was obtained from the crosses ♀B x ♂+ and ♀w x ♂+. The deviation from the 1♀:1♂ expected ratio was slightly significant (with an excess of ♀ over ♂) among the flies first hatched; however, in the grand total (when the F<sub>1</sub> was complete) the 1:1 segregation fitted well. These results were obtained from 190 cultures (small bottles), each started with a single pair of flies of the same mating type.

The backcross w/+ x w was used to find out if the observed differential rate of growth could also result from different genotypes within the same sex. This time w/+ females and + males were in excess among the flies first hatched. Similar results were obtained from the mating Pm/+ x Pm/+, where the heterozygous females and males in the F<sub>1</sub> hatched significantly earlier than normal segregants. The described phenomenon is rather variable for different mutants and matings. The rapid development of the heterozygotes compared to the hemizygotes and homozygotes varied from a minimum of 4 to a

maximum of more than 24 hours.

These observations, which are still in progress and need to be supplemented by more extensive data and a more critical statistical evaluation, seem quite interesting and promising. The existence of a phenomenon of this kind might, for example, throw some light on the abnormal maintenance of lethal genes, so frequently observed by students of population genetics.

Nolte, D. J. Homologous eye pigments and genes in species.

Histological and spectrophotometric studies of the eye pigments of D. melanogaster, D. simulans, D. opisthodelaina, D. pseudoobscura, D. persimilis, and D. miranda have shown that the red and brown pigments described for the first species are present in all these species. Small differences in the photometric curves of eye extracts will be described in a future publication. In the melanogaster group both pigments are present in the primary pigment cells, but in the obscura group only brown pigment is present in these cells. Larger amounts of pigment occur in D. opisthodelaina than in D. melanogaster, and the obscura group has less red but more brown pigment than the melanogaster group. The mutants bw of D. melanogaster and pr of D. pseudoobscura produce the brown pigment only, whereas the se mutants of both species produce a modified red pigment such as has been described for the former species.

Nolte, D. J. Missing multiple alleles.

Studies of the eye histology and pigments of ten series of multiple alleles of D. melanogaster have brought to light the effect of such mutations on the content of the red and brown pigments. Two new alleles have been discovered, car<sup>2</sup> and we<sup>3</sup>, and on the basis of the increases in content of pigment in the series of multiple alleles at the white and garnet loci it is postulated that some of the alleles at each of the loci have not yet been recognized. In both series the rise in content of red pigment is geometric and that of brown pigment is arithmetic, although the red and brown orders of alleles do not coincide within a series. For the red pigment in the white series, gaps appear between colored and eosin<sup>2</sup>, and between coral and satsuma; for the brown pigment, gaps appear between satsuma and eosin<sup>2</sup>, eosin<sup>2</sup> and wine, coral and colored. These are all presumptive alleles at or near the tops of the quantitative series, and if such alleles have relatively large amounts of both pigments they may possibly be difficult to distinguish from the wild type.

In the case of the garnet series, an allele g<sup>5</sup> seems to be missing, with more red pigment but less brown pigment than the allele g<sup>3</sup>. Its eye phenotype would probably resemble that of the mutant carnation.

Ogaki, M., and Tsukamoto, M.

It was reported in DIS-27 that resistance to DDT, BHC, parathion, chlordane, etc. is controlled by a dominant gene located near 70 on the second chromosome. Further experiments have been carried out to obtain more details, using multichromosomal or second-chromosomes mutant strains, such as cn; ca; gvl, cn; bar-3; gvl, and cn wt, and a multiresistant strain, Hikone-R. As a result of various backcrosses, it is obvious that the major gene for resistance to DDT and BHC is located at 66± on the second-chromosome map, at least in the larval test. Therefore, it is considered that DDT and BHC resistance may be controlled by the same gene or pseudoalleles.

Okada, T. Comparative morphology of the Malpighian tubes of adult drosophilid flies.

It was concluded from a comparative study of the adult Malpighian tubes of about 75 species of drosophilid flies that tubes with rather short common trunks and with the tips of the posterior branches separated from each other represent a primitive type in this family, whereas

tubes with comparatively long common trunks and with the tips of the posterior branches either fused or closely apposed are derived from the original type. Moreover, species with longer branches were found to have shorter common trunks.

Oshima, C. DDT resistance in populations of D. melanogaster.

The DDT resistance of adult flies in D. melanogaster populations composed of mixtures of a resistant strain (Fukuoka) and a nonresistant strain (Canton-S) was unexpectedly increased in the  $F_2$  generation. Although the resistance fluctuated in the following ten generations, it was not lost even in medium containing no DDT. When mixed populations which were heterogenic for DDT resistance were selected by DDT, their resistance increased as high as that of the original resistant strain. It is assumed that the DDT resistance, of adult flies at least, is due to polygenic characters which are reinforced by a dominant gene located on the second chromosome and a modifier on the third chromosome.

Oshima, C., and Taira, T. Population genetics of dimorphism in the color pattern of D. rufa.

Dimorphism in D. rufa was described by C. Oshima in DIS-26. This dimorphism may be recognized in the banding of the abdominal segments. The "dark" type has banding similar to that in the male melanogaster,

whereas the "light" type has banding like that in the female melanogaster; the dark marking is completely dominant over the light. Comparative studies were made of these types with respect to viability of larvae and adult flies, speed of development, the proportion of different genotypes in populations at equilibrium, differential advantage in mating, intra- and inter-competition, and variation in number of peg-like bristles on the egg-guide. In the laboratory population, there is a significant excess of dominant phenotypes. D/D and D/d males are found in copula significantly more frequently than d/d males. D/D and D/d seem to be somewhat faster in development than d/d in the same culture.

Oster, Irwin I. Modification of brown variegation by the Y chromosome in the female of D. melanogaster.

Variegation or mottling caused by the juxtaposition of genes located in euchromatic regions to heterochromatic regions has been found to be suppressed by the addition of extra Y chromosomes in the

male and female of D. melanogaster by Gowen (1933, 1934) and Dubinin (1935). The latter investigator presented some evidence indicating that the parts of the Y are not equally effective as regards their influence on mottling. With the aid of some of Professor H. J. Muller's special stocks, we have studied the effects of different parts of the Y chromosome on variegation. A series of crosses was made of y v f.; B1 bw<sup>VA</sup>/InNSL InNSR px sp females to males with different Y complements (these have been previously described in DIS by H. J. Muller and his associates). The  $F_1$  females of the composition, y v f.; B1 bw<sup>VA</sup> with no Y or containing the whole Y or part of it were examined, and the following results were obtained. (By "variegated" we imply

here not merely a nonuniform color but a color which averages distinctly lighter than that of vermillion. By "nonvariegated" we imply a color substantially like that of vermillion, although careful examination often discloses small speckles.)

$y\ v\ f.=/no\ Y; B1\ bw^{VA}$	variegated
$y\ v\ f.=/sc^{VI}Y^S; B1\ bw^{VA}$	partially variegated
$y\ v\ f.=/Y^{lc}; B1\ bw^{VA}$	very slightly variegated
$y\ v\ f.=/Y^S.Y^S\ #2; B1\ bw^{VA}$	nonvariegated
$y\ v\ f.=/Y^l; B1\ bw^{VA}$	nonvariegated
$y\ v\ f.=/scY^l; B1\ bw^{VA}$	nonvariegated
$y\ v\ f.=/sc^8.Y; B1\ bw^{VA}$	nonvariegated
$y\ v\ f.=/Y^+; B1\ bw^{VA}$	nonvariegated

It appears from these preliminary results that a whole long arm of the Y or 2 short arms are equivalent to the whole Y chromosome as regards the suppression of variegation in the female of D. melanogaster.

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Pantelouris, E. M. Effect of the ovary on the growth of the oviduct.

the ovary on the other side. It seems that oviduct growth requires an inducing stimulus from the ovary.

Pantelouris, E. M. Transplantations of larval ovaries.

which egg cytoplasm is not yet elaborated) between races selected for large and small body size. (1) Ovaries of third-instar Large line LZ 5 (homozygous for w) were transplanted into Small line ES 4 (+/+), and the offspring mated to LZ 5 ♂. Perfectly clear separation of offspring from transplanted and from host eggs was possible on the basis of eye color. The transplanted eggs showed no evidence of a maternal effect on size; nor was any found in the next generation. (2) Ovaries of Small line ES 4 transplanted into Large line LZ 5 (mated with ES 4) rarely gave offspring, and these showed no maternal effect. (One exceptional individual gave numerous offspring which were classified as homozygous Small, and these showed a considerable maternal effect; but it seems likely that the host larva was, by mistake, a Small-Large hybrid instead of pure Large). The chance of a transplanted ovary's attaching to an oviduct is somewhat improved if one host ovary is extirpated before the transplant is inserted, but the size of the ovary, and the yield of eggs, remains small. However, enough transplant offspring have been obtained to confirm the lack of an effect of the maternal bloodstream.

If, during the larval stage, the ovary on one side is removed, the lateral oviduct to which no ovary is attached fails to develop, although occasionally it grows and becomes attached to

Poulson, D. F., and Rizki, M. T. M. Spiracular glands in *Drosophila*.

*Drosophila: funebris, melanica, hydei, repleta, virilis*) and of *Zaprionis*

Unicellular spiracular glands have been observed by us in larvae of various species of *Drosophila* (subgenus *Sophophora*: *melanogaster*, *persimilis*, *prosaltans*, *willistoni*; subgenus

vittiger. These correspond very closely to the unicellular perispiracular glands which Keilin (1944, *Parasitology* 36: 1-66) described in larvae of a number of parasitic Diptera. There are at least two club-shaped unicellular organs associated with each posterior spiracle. The cytoplasm of these secretory cells shows a system of canaliculi which collect into a duct passing to the posterior region of the spiracle, presumably opening on the exterior surface. The secretion within the cells and their ducts is stainable with Sudan Black. This is in agreement with Keilin's conclusion that the glands are concerned in maintaining the hydrofuge properties of the posterior spiracles. It is possible that some larval lethals in which the tracheae are never fully filled with air (e.g., that described by Kaliss, 1939, and another by Poulson, 1940) may involve the malfunction or absence of these cells. A search is being made for such lethals. A detailed histochemical study of normal glands is in progress.

Rasmussen, I. E. Paper chromatography in the study of taxonomic relationship in the genus *Drosophila*.

Twenty different *Drosophila* species (Fam. *Drosophilidae*), *Zaprionus vittiger* (Fam. *Drosophilidae*), and *Aphiochaeta xanthina* (Fam. *Phoridae*) have been studied chromatographically in order to compare biochemical similarity with taxonomic relationship. Decapitated flies, washed in 95% ethanol and boiled for one minute in distilled water, were squashed on Whatman No. 1 filter paper for chromatography and developed in descending one-dimensional flow. Two solvents were used: (1) 2 parts n-propanol and 1 part 1% ammonia; (2) 4 parts n-butanol, 5 parts distilled water, 1 part glacial acetic acid.

The ninhydrin-positive patterns showed no differences among the various species of the *Drosophilidae* family, but a difference was found between *Aphiochaeta xanthina* and the members of the family *Drosophilidae*.

The fluorescent patterns, as revealed by ultraviolet light (B. L. lamp fixtures, 15 watts), showed that: (a) every species has a characteristic and constant chromatographic pattern, (b) different species can be differentiated by means of one or more spots, (c) the grouping of species according to biochemical similarity corresponds to the taxonomic groups established by Sturtevant on the basis of morphological characters, (d) some spots are suitable for distinguishing the established taxonomic levels--families, genera, subgenera, groups, and subgroups.

Redfield, Helen. Local differences in crossing over produced by two apparently like white alleles in *D. melanogaster*.

published data (not involving complications) relevant to this basic problem and they lead to uncertain conclusions: those of Serebrovsky (1927) deal with the influence of the gene purple (an increase in purple homozygotes) on black-cinnabar crossing over in the central region of II; and those of Prokofyeva-Belgovsky and Belgovsky (1943) show a decrease in the yellow-split region of the distal end of the X in white mothers as opposed to mothers homozygous for the wild-type allele of white. There were no special precautions in either set of experiments to control the internal genetic environment, and there is some question of control of the external environ-

A limited change in the constitution of crossing-over strands, such as accompanies the substitution in the homozygote of a mutant allele for the wild type, might be expected to lead to a localized change in crossover values. There are two sets of

ment also. The present crosses were made under conditions of more rigorous control. Two alleles of white were used in the isogenic lines established by Dr. Jack Schultz. These two new whites occurred separately in the inbred Oregon-R wild-type allele;  $w^{48h}$  occurred as a single male from X-rayed material,  $w^{51a}$  arose spontaneously as three males in a single-pair mating. The two are indistinguishable from each other and from the original standard white (w) both phenotypically and (as shown by observations of Dr. Jack Schultz and D. H. Hungerford) in the picture shown by the salivary-gland chromosomes. These alleles provide proper material for tests of the effects of homozygous genes, especially when used in conjunction with the booster influence of heterologous inversions. At 25° C in isogenic Oregon-R strains the yellow-split value (in the presence of the left Curly inversion and the Payne inversions) was  $11 \pm 0.5$  for the homozygous wild-type (Oregon-R) allele, and  $11 \pm 0.3$  for the  $w^{48h}/w^{48h}$  homozygote, but it was only  $6 \pm 0.4$  for the  $w^{51a}/w^{51a}$  homozygote. The difference between the value for  $w^{51a}/w^{51a}$  and the other two types is striking and clearly significant. The mutation to the white phenotype at this locus is in the one case, and is not in the other, correlated with a change in the crossing-over frequency in the immediate region. It is possible that the difference between these alleles is bound up with the problem of "duplicate" genes at the white locus, but there are alternative hypotheses which should be tested.

Ritzki, M. T. M. Secretory activity of the proventriculus of *Drosophila*.

Histological sections of the larvae of *D. melanogaster* and *D. willistoni* have been stained with the periodic acid-Schiff reagent (PAS). A stronger PAS reaction is found in an annulus four cells wide encircling the middle of the proventriculus than elsewhere in this region of the gut. These cells have not been differentiated from the remaining cells of the proventriculus by any of the other staining procedures which have been applied. The peritrophic membrane is strongly PAS-positive and gives a chitosan reaction for cuticle. It is secreted in the proventriculus and appears in that region of the proventricular lumen surrounded by this annulus. Pretreatment with various enzymes (saliva, diastase, amylase, hyaluronidase, pepsin, trypsin) followed by the PAS reaction did not affect the stainability of the intracellular granules in this annulus, the peritrophic membrane and the larval cuticle. Various changes in PAS-stainability were noted in other tissues after some of these pretreatments. The heavily staining intracellular granules in this annulus are either precursors of chitin or cuticular material which forms the peritrophic membrane.

Sandler, L. Heterochromatic exchange between reversed acrocentric compound X chromosomes and FR2.

Cytologically, in larval neuroblast cells, it appears as a rod-shaped chromosome about the size of the X with an extremely small short arm presumably derived from the short arm of the Y chromosome. From females carrying a reversed acrocentric compound X chromosome (= double X chromosome) and this fragment, occasional (approximately 1/250) breakdown X chromosomes are recovered. It had been assumed that these resulted from exchange between the long arm of the fragment and the interstitial heterochromatin of the compound chromosome. An analysis of certain of the breakdown products revealed, however, that in fact the exchange involves (apparently in all cases) the cytologically almost invisible short arm.

FR2 is a heterochromatic chromosome fragment (originally described by Novitski, Genetics 37: 231-283) genetically equivalent to the long arm of the Y chromosome and carrying the normal allele of y from sc<sup>8</sup>.Y (Muller, DIS-22: 73-74).

The situation in the particular case analyzed was as follows. The basal arm of the reversed acrocentric compound was  $In(1)sc^8$  but carried no  $y$  locus, while the distal arm was in normal sequence and marked by  $y$ . The centromere had  $y^L$  carrying  $y^+$  attached to it. Females carrying this compound and FR2 were crossed to  $y$  B males. Breakdown products, recovered as  $B/+$  females, would, on the assumption that crossing over occurred between the long arm of FR2 and the interstitial heterochromatin of the compound, be expected to be both  $y$  and non- $y$ . A rather large number of such breakdown chromosomes were collected, but all were non- $y$ . This led to a consideration of the expectations if the exchange involved the short arm of FR2. From such an exchange there should be produced chromosomes in both normal and inverted sequence, and both types should have  $y^L$  attached to the centromere. Of 16 products tested, 4 proved to be inverted and 12 were in normal sequence. All but one (which probably lost  $y^L$  in stock) were fertile with  $Y'$ ; sterile with  $Y^{cl}$ ; and viable as  $XO$  males. Moreover, on the assumption that the exchange involved the short arm of FR2, the chromosomes in inverted sequence should not carry a  $y$  locus distally. The crossover data indicate that indeed they did not. Finally, under this assumption, the breakdowns in normal sequence should have a cytologically long arm ( $y^L$ ) attached to the centromere. An examination of one of the products in normal sequence revealed that this was the case.

This represents additional evidence for the view reported by others that in the case of heterochromatic exchange between the X and Y chromosomes the arm always or almost always involved is  $y^S$ .

Sävhagen, Ruth. Studies on the relation between X-ray-induced recessive lethals and chromosome aberrations in *D. melanogaster*.

between these two frequencies, however, was not as big as the ratio between the corresponding rates of chromosome breaks. Lüning suggested that the higher rate of recessive lethals in sperms from the 7th to the 10th day was due to breaks and that the rate of break-independent, intragenic changes was not changed. In order to verify this, the following experiment was carried out.

Males from a yellow stock,  $y^{16}$ , which had appeared in an X-ray experiment in this laboratory, were irradiated (1000 r) when 0-1 day old and were subsequently mated to Muller-5 females. Eggs were collected on the 1st-3rd day after irradiation. On the 7th day the males were mated to new virgin Muller-5 females, from which eggs were collected for 4 days. The  $F_1$  daughters from these two series were tested in the usual way. If 10 or more Muller-5 males but no  $y^{16}$  males appeared among the  $F_2$  offspring, the  $y^{16}$  chromosome was classified as containing a lethal.

The recessive lethals were balanced by the Muller-5 chromosome. By crossing over tests the lethals were mapped. For cytological testing,  $y^{16}$   $1/M\cdot5$  females were mated to  $y^{16}$  males from the stock. Individuals homozygous for the  $y^{16}$  chromosome could be distinguished from their sisters by yellow teeth. The results are presented in the table. In the 7-10-day series some stocks were lost before  $F_3$  or  $F_4$ . In spite of the low number of tested lethals one sees that the rate of recessive lethals associated with chromosome aberrations is strongly affected by the rate of breaks (which according to

In a series of experiments, Lüning (1952) showed that the rate of recessive lethals induced in spermatozoa ejected within the first 6 days after irradiation was lower than in spermatozoa delivered from the 7th to the 10th day after treatment. The ratio

Time of insem. in days after treatment	Total number tested X chromosomes	Rec. leth.		Genetically and cytolog. tested rec. leth.	Rec. leth. with chromosome aberrations	
		No.	%		No.	%
1-3	1959	42	2.14	41	4	9.76
7-10	1581	61	3.86	50	20	40.00

Lüning is considered to be about four times higher in the latter series than in the former). The rates of recessive lethals which are not associated with chromosome aberrations are not statistically different. Hence these results confirm Lüning's hypothesis mentioned above.

Schalet, Abraham. The mutagenic action of 1,2-propylene oxide and ethyl sulfate on mature sperm.

Following postcopulatory vaginal douches of Basc females with 100% 1,2-propylene oxide and with low (2%, 5%) and high (33%, 50%, 100%) concentrations of ethyl sulfate dissolved in either 95% ethyl alcohol or absolute ethyl ether, the X-chromosome recessive lethal mutation rate in mature sperm of the Oregon-R stock was determined. Rapoport, after adding these substances to the food of developing individuals, obtained a mutation rate of "seven times the control rate" for the former substance and of 2.4% (22/911) for the latter. The mutability shown by mature sperm in our experiments was 1.2% (13 lethals, from 12 different males, in 1074 tests) for propylene oxide and 0.7% (6 lethals, from 3 different males, in 865 tests) for the low and 2.9% (34 lethals, from 16 different males, in 1184 tests) for the higher concentrations of ethyl sulfate. Oregon-R mature sperm have shown a low spontaneous-mutation rate (1 lethal in 1650 tests) in our other work. Thus the present work adds two more chemicals to those--formaldehyde and nitrogen mustard--shown to be mutagenic when applied to mature sperm.

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Scossiroli, R. E., and Rasmussen, I. E. Paper chromatography of interspecific hybrids in *Drosophila*.

Since *Drosophila* species studied by means of paper chromatographic technique were shown to have characteristically different fluorescent patterns, the same technique has been applied to interspecific hybrids. The purpose of this study was to determine whether at the fluorescent level it is possible to identify in hybrids not only the substances peculiar to both the parental species but also new substances peculiar to the hybrids, such as have been found in some hybrid birds by Irwin, using agglutination tests.

Using propanol (2 parts) with ammonia (1 part) as the solvent, the interspecific hybrids tested (spinofemora x setifemur, melanogaster x simulans, pseudoobscura x persimilis, pseudoobscura x miranda, nitens x lebanonensis) showed in the fluorescent pattern all the spots peculiar to both the parental species. Some of the spots were found to be increased in size and intensity of fluorescence over those observed in the parents ("biochemical heterosis"). In two-dimensional chromatograms of the hybrid D. melanogaster x D. simulans,

obtained using butanol-ammonia as the second solvent, a similar phenomenon of "biochemical heterosis" was noted, and it was also possible to identify new substances not shown by the parental species.

Sinoto, Y. Artificial isolation of salivary nuclei by a fluorochrome, acridine orange.

Salivary nuclei of *Drosophila* and *Chironomus* are easily isolated or freed from the cytosome by adding acridine orange solution and pressing slightly on the cover glass. The isolated, roundly swollen nuclei shine bluish green under ultraviolet radiation. The size of isolated nuclei of *Drosophila* (*D. melanogaster* and *D. hydei*) is about one-half that of *Chironomus*. The small red-orange body which often appears in the isolated swollen nuclei is a basic structure of nucleolus in *Chironomus* and of chromocenter in *Drosophila*. Salivary nuclei can also be isolated by acridine yellow, though the proportion of isolation is less than that with acridine orange.

Sobels, F. H. Mutagenicity of dihydroxymethylperoxide.

Dickey et al. (P.N.A.S. 35: 581, 1949) found that mixtures of hydrogen peroxide and formaldehyde are mutagenic in *Neurospora*. In

*Drosophila*, the mutation rates observed after injection of this mixture were similar to those produced by formaldehyde alone. This result is probably due to a high catalase activity in the fly, causing a rapid breakdown of the hydrogen peroxide. Dickey et al. suggested that the active principle is dihydroxymethylperoxide ( $\text{HO}-\text{CH}_2-\text{O}-\text{O}-\text{CH}_2-\text{OH}$ ), a compound which can be synthesized by combining formaldehyde and hydrogen peroxide (cf. Wieland and Wingler, Ann. Chem. 431: 301, 1923). Solutions of this peroxide were made with 0.7% NaCl and adjusted to a pH of 6.6 with a Sørensen solution. After injection of 0.20 mm<sup>3</sup> of a 0.06 molar solution into the abdominal cavity of Oregon-K males, tests were made for sex-linked lethals, using the Muller-5 method. Data of the preliminary experiments reported here suggest that in *Drosophila* dihydroxymethylperoxide acts as a weak mutagen.

Expt.	Mortality in %	n chromosomes	n lethals	lethals in %
P <sub>3</sub>	45	307	7	2.4
P <sub>6</sub>	67	355	4	1.1

Sobels, F. H. Mutation tests with formaldehyde injected into larvae and pupae of *D. melanogaster*.

Investigations of Auerbach (Z.I.A.V. 85: 479, 1953) showed that when formaldehyde is fed to *Drosophila* larvae, mutations are preferentially induced in the auxocyte stage. Formaldehyde injected into flies,

on the other hand, produces highest mutation rates in mature sperm. As feeding experiments with flies were not successful (Auerbach), it was thought of interest to study the effect of formaldehyde injected into the larvae. Mid-third-instar larvae (Oregon-K) were injected by a technique adapted from that of Ephrussi and Beadle. Accurately known quantities can be injected by placing two enamel-paint datum marks about one cm apart on a Pyrex needle with an internal diameter of 35 microns. The injected volume can then be calculated by measuring the internal bore at the upper and lower points. After the whole injection apparatus has been filled with water a small bubble of air is sucked into the needle, thus preventing the water and mutagen from coming

into contact and forming an easily observed mutagen-air surface from which measurements of the quantities injected can be made. Larvae were injected with  $0.013 \text{ mm}^3$  and pupae (6-8 hours after puparium formation) with  $0.02 \text{ mm}^3$ . The formaldehyde solutions, made up with 0.7% NaCl, were stained with a 0.2% methylene blue solution. The staining facilitates the location of fluid in the needle and enables one to observe whether any fluids escape after the injection. Pilot experiments with adults showed that methylene blue is not mutagenic by itself (table, third row) and that it does not deprive formaldehyde of its mutagenic activity (table, top rows). Tests for sex-linked lethals were made by means of the Muller-5 method. The sensitivity pattern in the testes of the treated males was studied by mating them to three fresh virgin females at time intervals of three days. The spontaneous-mutation rate in the Oregon-K stock only rarely exceeds 0.3%. The data are summarized in the table. They show that all the experiments with larvae and pupae, despite the wide concentration range used, were consistently negative.

Experiment	Concentration formaldehyde (%)	Sex-linked lethals							
		Days after treatment							
		1-3		3-7		7-10		10-13	
		n	l	n	l	n	l	n	l
Flies injected only formaldeh.	0.20	1152	29	466	4	307	-	535	10
		2.5%							
Flies injected + methylene blue (0.2%)	0.20	592	13	588	2	462	5	823	3
		2.2%							
Controls:									
methylene blue (0.2%)	-	392	1	-	-	-	-	-	-
Larvae injected	0.20	651	2	691	3	575	1	407	1
+ methylene blue (0.2%)	0.45	315	-	215	1	56	-	42	-
	0.65	355	2	278	-	146	-	-	-
	0.85	160	2	69	-	-	-	-	-
	0.90	630	1	590	1	-	-	-	-
	1.00	223	-	71	-	-	-	-	-
	1.20	668	3	613	-	362	-	-	-
Pupae injected	0.25	418	1	423	1	337	1	209	-
+ methylene blue (0.2%)	0.75	122	-	46	-	78	-	68	-
	0.90	266	1	361	-	240	1	208	-
Larvae Vapor.									
16 hours,	2.5	576	5	693	-	651	1	505	1
25° C		0.9%							

A possible reason for this result may be that the larvae do not allow greater quantities of fluid to be injected. In the experiments with flies,  $0.17 \text{ mm}^3$  of a 0.2% formaldehyde solution proved most active, whereas the larvae received only  $0.013 \text{ mm}^3$ . It is therefore possible that the formaldehyde concentrations in the larvae were not sufficiently high to produce mutations.

The negative data of these experiments are reported because they may be helpful in preventing a repetition of effort by other workers (cf. Oster, DIS-26: 116, 1952). (Work carried out under a British Council Scholarship at the Institute of Animal Genetics, Edinburgh.)

Spiess, E. B., and  
Schuellein, R. J. Fecundity,  
 egg hatchability, develop-  
 mental rates, and relative  
 survival of gene arrangement  
 carriers in D. persimilis.

D. persimilis from 8000' elevation in the  
 Sierra Nevada of Yosemite National Park are  
 being tested for relative survival of three  
 principal gene arrangements of the third  
 chromosome--Whitney, Klamath, and Mendocino--  
 first in population cages to determine net  
 survival and secondly by testing physiologi-

cal properties of flies carrying all combinations of the arrangements under optimal as well as crowded conditions, to determine if possible those factors which contribute to the net fitness of the genotype.

Relative survival of any two gene arrangements is obtained by the population-cage technique. For each pair of arrangements two cages are being run, with reciprocal initial frequencies of the arrangements. Indications are, after five generations at 15° C, that in both cages containing WT-KL the WT is superior to KL; but whether the heterokaryotype, WT/KL, is superior in net fitness to Wt/Wt or KL/KL is yet to be determined. Also, WT is superior to MD, but equilibrium has not yet been reached in any of the cages.

Tests to ascertain whether any physiological traits can be constantly identified with these gene arrangements have been carried out as follows. Strains of flies are maintained by mass mating. Strains containing identical chromosomal variants are cross-mated for homokaryotic combinations. Heterokaryotic combinations (WT/KL and WT/MD) are either the F<sub>1</sub>'s of strain crosses or the progeny obtained by crossing homokaryotic strain-cross F<sub>1</sub>'s. Flies to be tested were raised as follows. Strain "A" females and strain "B" males were mated in large bottles, about six pairs per bottle, with plastic spoons containing a small amount of fly food inoculated with bakers' yeast suspension. Spoons were changed every day for a week. Eggs which had been laid were transferred as follows. For fecundity tests, spoons with no more than 100 eggs were put into a large culture bottle containing food at 15° C; for hatchability and developmental-rate studies, eggs were collected from the spoons, washed in 70% alcohol, and planted on an agar surface in Petri dishes (see Technical Notes for composition of agar medium used). For each chromosome combination, four strains were mated in combinations 1 x 2, 2 x 3, 3 x 4, and 4 x 1. These four crosses were designated series A, B, C, and D respectively (see Table 1). Data were recorded as described by Spiess (Evol., 1952); fecundity in eggs per day.

Table 1

Karyotype	Series	1st ten days	2nd ten days	3rd ten days	Wtd. Av. 30 days
WT/WT <sub>2</sub>	A	20.9	(28)	21.7	(24)
	B	18.8	(24)	14.5	(22)
	C	19.0	(8)	14.0	(7)
	D	22.4	(18)	20.1	(18)
	Mean wtd.	20.42	(78)	*	*
KL/KL <sub>2</sub>	E	17.8	(20)	18.7	(17)
	F	19.8	(24)	17.5	(17)
	G	17.8	(19)	17.0	(15)
	H	18.6	(10)	17.2	(10)
	Mean wtd.	18.15	(74)		18.12

WT/KL....Not completed; 1st ten days range, 17.5-21.1 eggs/day.

\*Analysis of variance gives significant F value, i.e., no true mean for all four series.

Mean standard errors will be worked out at the completion of the experiment.

There is no apparent difference between the egg-laying capacities of WT and KL females. Heterozygotes do not show any signs of heterosis at present writing. These results are in marked contrast to those obtained with WT/KL from Jacksonville, California (elevation 800') raised under crowded conditions. The factor of crowding on these high-altitude strains is to be tested.

Table 2

Karyotype	Egg Hatching (%)	1st Instar Days	2nd Instar Days	3rd Instar Days
WT <sub>1</sub> /WT <sub>2</sub>	86.0-87.9	3-3.5	3.0-4.0	8-16
KL <sub>1</sub> /KL <sub>2</sub>	80.0-89.2	3.1-4.1	3.5-4.0	6-10
WT <sub>1</sub> /KL <sub>1</sub>	89.6-97.7	2.5-4.2	2.5-3.0	5-10
WT <sub>1x2</sub> /KL <sub>1x2</sub>	93.4-98.7	3.1-4.6	2.5-3.2	9-16

	Pupa Days	Total Days from Egg to Eclosion
WT <sub>1</sub> /WT <sub>2</sub>	13-16	28-40
KL <sub>1</sub> /KL <sub>2</sub>	12-15	25-38
WT <sub>1</sub> /KL <sub>1</sub>	10-14	22-35
WT <sub>1x2</sub> /KL <sub>1x2</sub>	13-18	24-41

All samples consisted of at least eight Petri dishes, for each series of which there were four per karyotype. Each Petri-dish culture contained 50-100 eggs. Note (Table 2) that WT does not differ from KL in hatchability, but that the latter's life cycle is markedly shorter owing to third-instar and pupal survival. Heterokaryotypes obtained by crossing a strain of WT to a strain of KL may show heterosis owing to heterozygotic loci on chromosomes other than the third, especially if the strains have been partially inbred in the laboratory. Nevertheless, if WT strain-cross F<sub>1</sub>'s are mated to KL strain-cross F<sub>1</sub>'s and evidence of heterosis still remains, heterotic loci on other chromosomes cannot account for the effect, in any degree. Per cent hatchability of eggs and rate of second-instar development, then, can be attributed to increased survival value contributed by heterotic loci on the third chromosome.

Takada, H. Occurrence of melanderi group and quinaria group in Hokkaido.

Four specimens (2 ♀, 2 ♂) of the melanderi group were collected on Mt. Taisetsu by net and banana traps. On the basis of external morphology and male genitalia, this species

appears to be quite closely related to D. pallida Zettersted, 1847, according to the description of Burla (1951). Male genitalia: Lower portion of genital arch has 30 bristles, upper portion 6; heel pronounced. Teeth on under surface of anal plate along lower outer margin, two large teeth at corner. Primary clasper has a row of primary teeth, about 8-10, and a number of stout marginal bristles, about 12. Secondary clasper has a straight row of 7 teeth.

Six male flies of the quinaria group were collected on Mt. Taisetsu, and at Bihoro and Akan, by means of banana traps. This species is quite closely related in many external characters to D. kuntzei Duda, 1924, according to the description of Burla (1951). Male genitalia: Genital arch has 8 bristles on

middle and lower portion, arranged in a semivertical row, heel present; toe low, not covering the clasper. Anal plate separated, with rear angle slightly developed. Clasper, one, broad; primary teeth, 12-15; secondary teeth, 4-6; very long marginal bristles, about 6-8.

Tantawy, A. O. Genetic variability of wing length in different selected lines.

Three estimates of heritability of body size--that is, wing and thorax length--in D. melanogaster have been carried out in an initial foundation population, using the regression method. Two of these estimates

involved random matings and the other assortative matings, the last being corrected for the magnified variance between the parents. The results are as follows:

Test	Type of mating	Heritability (%)		Degrees of freedom	Genetic correlation (%)
		Wing	Thorax		
1	Random	41±4	46±5	98	84
2	Random	47±4	48±3	98	78
3	Assortative	45±6	47±6	98	83

The weighted means for all tests indicate that nearly 45% and 47% of the total variance for wing and thorax length, respectively, are apparently due to additive gene effects. Heritability estimates clearly demonstrate the presence of abundant genetic variability for both the characters in the initial stock.

Genetic correlation between wing and thorax length was calculated in the first two tests by Hazel's formulae and in the third test by Reeve's formulae. After correction for the magnified variance between parents in the third test, the genetic correlation was found to be 81.7%, indicating a highly positive estimate between the two characters in the initial foundation stock.

Selection for long and short wing length was carried out, using three different systems of mating in maintaining the various selected lines: brother-sister, double first cousins, and outbreeding. In each system two parallel selected lines were maintained, one selected for long wing and the other for short wing. Selection was effective in all systems of mating from the first generation, as reported earlier (DIS-27: 115); short-wing selected lines displayed greater response to selection than long-wing selected lines. Thorax length in all the selected lines showed the same response to selection as wing length. Heritability estimates for wing length were calculated at the fifth, tenth, and fifteenth generations in the case of brother-sister and outbreeding systems. The results are shown in the following table.

Generation	Brother-Sister		Degrees of freedom	Outbreeding		Degrees of freedom
	Long	Short		Long	Short	
5	19.4±6	23.2±7	18	34.0±2	43.4±8	18
10	6.2±4	10.3±6	18	32.1±5	59.8±10	18
15	4.3±4	7.6±4	15	41.3±6	45.4±8	16

In the case of the various inbred lines, heritability estimates were calculated at the 25%, 50%, and 75% coefficients of inbreeding. The results

are as follows:

Coef. of inbreeding (%)	Brother-Sister		D.F.	Double first cousins		D.F.	Expected decline
	Long	Short		Long	Short		
25	24.5±4	30.3±5	18	41.5±5	48.6±6	18	38.0
50	21.2±4	27.8±6	18	42.9±4	40.2±7	16	29.0
75	15.1±5	20.2±3	17	34.2±5	32.6±4	17	17.0

Heritability for wing length shows that the genetic variability has decreased in the inbred selected lines maintained with brother-sister matings almost to the expected decline. Selected lines maintained under an outbreeding system do not show any reduction in the heritability estimates.

At the same coefficient of inbreeding the various inbred selected lines with different intensities of inbreeding show different results in the heritability estimates. Brother-sister matings show almost the same values as the theoretical expectation, whereas double-first-cousin matings cause a little reduction.

Brother-sister matings automatically tend to reduce heterozygosity, and by the time animals have reached the fifteenth generation they are about 96% homozygous. At this level of homozygosity selected inbred lines show heritability estimates between 4% and 7% for long and short wing length, respectively. Genetic variance at this level of inbreeding should have been reduced to about 3% according to the start point. Thus the reduction of the heritability estimates expected from the known rate of inbreeding is not far beyond that actually observed. Matings between double first cousins cause a slight reduction in the heritability estimates, more than the expected decline. These results clearly indicate that the more intensive inbreeding, accompanied by selection, was more effective in decreasing the genetic variability within inbred lines than the milder rates.

Genetic correlation between wing and thorax length, which is the description of the relationship between the additive deviations for the genes for the two characters, is found to be high in the initial foundation population. It remains, in general, stable in the case of double-first-cousins mating at the lower level of inbreeding, and declines slightly at the higher levels; whereas matings between sibs show more reduction, particularly at the 75% coefficient of inbreeding. At this level, estimates of such genetic correlation are found to be 58%, 57% and 73%, 66% in the case of brother-sister and double-first-cousin matings, for long and short wing length, respectively. Although this genetic correlation is calculated as 31% and 32% in the long-and short-wing selected lines, respectively, with brother-sister matings at the level of 96% of inbreeding, the differences are probably not significant. As the genetic variability is reduced by inbreeding, we would expect the genetic correlation to be reduced also, and this was found to be the case in the selected inbred lines with brother-sister matings.

In the case of outbred lines, genetic correlation remains almost constant.

Telfer, J. D., and Abrahamson, S. The production of sex-chromosome loss and dominant lethals in *Drosophila* sperm by fast neutrons.

Baker and Von Halle (1953) were the first workers to show that X-rays produce significantly different dominant-lethal frequencies in sperm used the first and second days after treatment. These results were corro-

borated both by our group (1954) and by Lüning (1954). Using fast neutrons, however, Baker and Von Halle (1954) observed that differential survival rates of eggs were not obtained. Our present work, performed under the guidance of Dr. H. J. Muller, not only confirms their results but also extends this phenomenon of fast neutrons to loss of chromosomes and chromosome parts, as detected by the production of exceptional males, lacking the paternal Y or X or its marked ( $y^+$ ) portion.

The irradiation was performed through the courtesy of Dr. Alexander Hollaender, Dr. C. W. Sheppard, and others at the Oak Ridge National Laboratory, using the 86-inch cyclotron. The dosimetry was carried out by Dr. Sheppard and his staff.

Nonvirgin males having their X chromosome derived from the Oregon-R stocks and having a Y of  $sc^8.Y$  type (marked with  $y^+$ ), 2 1/2-3 1/2 days old, and  $Xc2/sc^8.Y$  males, 2 1/2-4 1/2 days old, were simultaneously irradiated with 800 rep (246 n units) of fast neutrons. Immediately after the irradiation the males were mated for 24 hours to virgin females of  $yX.Y/yX.Y$ ;  $st/st$  genotype, the X chromosome having been the noninverted one, ( $Ins24L + A2Ry$ ), with  $Y^S$  at the left end and  $Y^L$  at the right, without a scute-8 attachment, obtained by Novitski (DIS-25). At the end of this time the males were removed and given new virgin females of the same type for an additional 24-hour period.

A portion of the females from each group containing the sperm from the  $Xc2$  males were used for dominant-lethal studies. Eggs of the first 4 days of laying after the insemination period were used in these studies. In some of them, single-pair matings were used, to avoid the possibility of using females which had failed to copulate or which had been sterile or had mated with a sterile male. That this happened very rarely, however, was shown by the substantial agreement between the egg mortality in the fertile-pair matings and in the mass matings (see table) and by the fact that not one single-pair mating was sterile from one of these causes among the 21 tested. However, 5 of the 21 tested are not included because of death of the females.

#### Exceptional yellow males

<u>Oregon-R/<math>sc^8.Y</math></u>	<u>800 rep Neutrons</u>	<u>Control</u>
1st 24 hr. sperm	1.01% (31 $y^+$ /3034 $y^+$ )	0.13% (1/772)
2nd 24 hr. sperm	0.89% (33/3663)	0.0% (0/1668)
<u><math>Xc2/sc^8.Y</math></u>		
1st 24 hr. sperm	2.20% (253/11,984)	0.83% (25/3021)
2nd 24 hr. sperm	2.24% (239/10,782)	0.81% (25/3077)

#### Dominant lethal frequency (corrected for controls)

<u><math>Xc2/sc^8.Y</math></u>	<u>800 rep Neutrons</u>	<u>No. eggs counted</u>
<b>Fertile single-pr. matings:</b>		
1st 24 hr. sperm	58.94%	1,837
2nd 24 hr. sperm	56.01%	2,529
<b>Mass matings:</b>		
1st 24 hr. sperm	54.58%	7,538
2nd 24 hr. sperm	55.53%	10,954

The remaining females of each insemination period were allowed to oviposit for nine days--that is, through three broods, each of three days duration. The  $F_1$  were examined for yellow male exceptions. These are produced by complete loss of either the X or the  $sc^8.Y$  chromosome, large deletion of the X, or partial loss or mutation of the Y involving the  $sc^8$  attachment. The phenotype of the regular  $F_1$  males is gray ( $y^+$ ).

Although the frequency of yellow males from the  $Xc2$  stock is presented in the table, it is not as reliable an index of neutron effects as is that of the Oregon-R stock, because the  $Xc2$  controls show a higher frequency, with greater variability, owing either to increased spontaneous breakage of the  $Xc2$  chromosome or to an increased frequency of imperfect disjunction with the  $sc^8.Y$  chromosome.

(Work supported by grant from the Atomic Energy Commission, Contract AT(11-1)-195.)

Thoday, J. M., and Boam, T. B.  
Selection for increase of sternopleural bristle number.

An experiment is being run to determine whether by suppressing selection for normal wing development, using  $dp$  in one line and  $vg$  in another (both started as new  $F_2$  segregants) and selecting for increase of sternopleural bristle frequency, it is possible to so disturb the normal genetic balance that  $F_1$ 's between the lines show significant disturbance of wing development. So far, after more than a year, no such effect has resulted. However, it is expected that much longer will be required, and furthermore the  $vg$  line has proved highly resistant to selection so that the results are by no means conclusive. The lack of response of the  $vg$  line is interesting. It is not due to rapid establishment of homozygosity, for there is still genetic variance in the line. It was thought to be due to selection for increase of bristle number resulting in more extreme expression of  $vg$ , hence inviability. Extraction of  $F_2$   $vg$  flies from the  $F_1$  with the  $dp$  line (using  $F_1$  flies selected for high bristle number) gave  $F_2$   $vg$  with no more bristles than the parental  $vg$  line. However, a line established from these responded rapidly to selection. Tests on this showed the selection to have established a high frequency of  $dp$   $vg$  chromosomes, but the flies without  $dp$  also had a higher bristle number than the main  $vg$  line. An extracted line free of  $dp$  is being established, and tests will also be made to determine whether poor response to selection for sternopleural bristle number is a characteristic of  $vg$  homozygotes.

A further interesting result, which is to be followed up, is the observation that asymmetry of bristle number is low in the  $F_1$ 's between early generations, but that this relative homeostasis is rapidly lost with selection for bristle number in the parent lines. At present it looks as if  $F_1$ 's are again becoming better balanced than the parent lines.

Thompson, P. E. Fourth-chromosome crossing over in CMI heterozygous flies.

Matings of  $sc^{S1} B$   $InS w^a$   $sc^8/+$ ;  $C$   $Sb/+$ ;  $ci$   $ey^R/+$  females with  $ci$   $ey^R$  males resulted in a progeny of 392 flies among which 4 showed crossing over between  $ci$  and  $ey^R$ , a crossover percentage of 1.02. The crossover types were given absolute identification by progeny testing. All progenies were hatched at  $18^\circ C$  to enhance the expression of cubitus-interruptus.

Thomson, J. A. Population-cage competition between *w* and *w<sup>e2</sup>*.

After four months at 25° C, the frequency of *w*, competing in a population cage with *w<sup>e2</sup>*, was found to have dropped from 0.5 to 0.28. Selective mating tests in which

*w/w<sup>e2</sup>* heterozygotes were given the choice of mating with *w* or *w<sup>e2</sup>* males showed that female mating preference was the major factor responsible for the observed decrease in the frequency of the *w* allele.

Attempts to determine allele frequencies, using adults removed from the population cage by phototropism, failed to give consistent results. This was found to be due to diurnal periodicity exhibited by the flies and also to the presence of genotypic gradients within the cage. Erroneous results could be obtained by calculating the frequencies of sex-linked genes on the basis of male numbers in adult samples taken by this method.

In tests where adults were removed by their phototropic response from a vessel containing equal numbers of *w* and *w<sup>e2</sup>* males, significantly fewer *w* males were recovered than *w<sup>e2</sup>*. Estimates of the total populations of population cages by dilution methods, using genetically labeled and sterilized males, were found to be invalidated by these difficulties in collecting adult samples.

Tsukamoto, M., and Ogaki, M.  
Nicotine resistance.

Since the multi-resistant strain Hikone-R has a higher level of resistance to nicotine sulfate than various other strains

tested in our laboratory, this strain was reared with food medium containing 0.06% nicotine sulfate for fifteen months or more to increase the level of its resistance. Then a genetical investigation was carried out, using *bw*; *st*; *sv<sup>n</sup>* as a susceptible mutant strain, by the same method as that used for analysis of DDT and BHC resistance.

The results of backcross experiments with a multichromosomal mutant strain, *bw*; *st*; *sv<sup>n</sup>* ♀ x *F*<sub>1</sub> (Hikone-R ♀ x *bw*; *st*; *sv<sup>n</sup>* ♂) ♂, distinctly indicated that the nicotine resistance of the Hikone-R strain is probably controlled by one dominant gene in the third chromosome. Preliminary tests suggest that this nicotine resistance-gene is located in the middle part of the third chromosome, probably near *Sb* or *ss*.

We have bred another resistant strain, Nicotine-R, derived from mixtures of 14 Japanese wild stocks other than the Hikone-R strain, with successive selections for resistance to nicotine sulfate. The level of nicotine resistance of this strain has greatly increased; it is about the same as that of Hikone-R strain at the present time. A genetical analysis is being carried out with this strain.

Ulrich, Hans. Difference in radiosensitivity of nucleus and cytoplasm of *Drosophila* eggs.

The single-hit type of dose-effect curve for killing of *Drosophila* eggs by X-rays before cleavage, and the maximal radiosensitivity of the nucleus-containing second

fifth of the egg at that stage, demonstrated the decisive importance of the nucleus in the lethal effect of irradiation (DIS-27, pp. 117-118). In order to obtain quantitative data concerning the relative sensitivities and the dose-effect curves of nucleus and cytoplasm, we irradiated the anterior or the posterior halves of 10-20-minute eggs with different doses of X-rays (50 kv, 10 ma, 1 mm Cellon, FD 40 or 10 cm, about 600 to 96,000 r per min.), while shielding the other halves with lead. The dose-effect curve resulting from irradiation of the nucleus-containing anterior half corresponds well as to

shape, slope, and dose/percent values (single-hit type,  $D_{50}$  about 450 r) to that obtained when eggs at the same age are totally irradiated. In comparison, irradiation of the posterior half, which at the stage in question does not contain any nucleus, yields a dose-effect curve of a similar shape (not yet exactly determined on account of the difficulty of obtaining sufficient data in partial X-raying), but at a much higher dose-level ( $D_{50}$  about 30,000 r). This indicates that the radiosensitivity of the cytoplasm is about 66 times lower than that of the nucleus. Probably the difference is still greater, for, considering the method used so far, the very high doses that were applied to the posterior half, and the significantly higher sensitivity of the anterior half, it seems likely that a considerable proportion of the lethality caused by irradiation of the posterior half of the eggs may have been due to scattered X-rays, which reached and affected the nucleus shielded by lead. Another and more interesting question important to our problem concerns the possible action proceeding from the irradiated posterior half to the nucleus in the shielded anterior half through secondary electrons or diffusing ionization products.

Waddington, C. H. Genetic assimilation of the bithorax phenotype.

Starting from an Oregon wild-type stock, eggs 2 1/2-3 1/2 hours after laying were given ether vapor treatment for 25 minutes. Adults showing a bithorax phenotype were used as parents of the next generation ("upward selection line"); a downward selection line was run from those remaining wild-type. Two replicates were made (Expts. I and II). Progress was made in both directions of selection, but cannot be measured precisely owing to high mortality of extreme bithorax phenotypes in the upward lines. After 8 generations of upward selection in Expt. II, one untreated fly showed a slightly enlarged haltere, and in the next generation there were 10 of these. From these a number of "haltere effect, HE" stocks were bred. The best of these throw up to 80% of flies with enlarged halteres, but the character seems to be due in the main to a recessive lethal with dominant HE effect, which acts as an allele of *Exl*.

At a later stage, about generation 29, an apparently identical gene arose in the upward-selected line of Expt. I. These two appearances of inherited haltere effect appear to be due to the spontaneous occurrence of new mutations (although the possibility that the second was a contamination cannot be excluded). The number of flies examined per generation was of the order of 3000.

At generation 29 in Expt. I a few untreated flies were also found to show not only enlarged halteres but small pieces of thorax-like material formed from the metathoracic bud. From these HE lines were derived showing this phenotype in frequencies up to nearly 100%. Crosses to marker stocks show that the character is polygenic, all chromosomes being involved. Crosses with wild types, and backcrosses of the *F*<sub>1</sub>, give much higher percentages of bithorax phenotypes when the mother is HE, indicating that the genes act by a maternal effect on the egg structure. This condition may well be due to the bringing together, by selection, of genes which were already present in low frequency in the initial population.

Waddington, C. H., and Bateman, K. G. Specificity of sensitivity to pheno-copying agents.

There is no close connection between sensitivity to ether treatment of eggs (producing bithorax phenocopies) and heat treatment of pupae (producing crossveinless phenocopies). Both high and low HE lines were slightly more sensitive to crossveinless treatment than the original wild stock, but a stock

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selected for sensitiveness to heat was less sensitive to ether than the wild. The same heat treatment to pupae may produce several different phenocopies (e.g., absence of veins, extra venation, etc.), and it is possible by selection to build up races which produce only one type of phenocopy in high percentage and the others in low numbers.

Weltman, A. S. Nonrandom disjunction in attached-X females.

unequal arm length were formed by the cross of triploid sc v f.f v y/FR1 y m car females by y B (YSX.YL) males. The FR1 y m car chromosome is one of normal sequence, with Y<sup>S</sup> located at the distal end (Braver).

Three attached-X females were obtained. Two carried the mutants sc v f on one arm, and y m f plus distal Y<sup>S</sup> heterochromatin on the other arm. A third had sc v f on the shorter arm, and the markers y m, plus the distal Y<sup>S</sup> on the longer arm. The first two are designated as lines 1 and 5, and the third as line 6.

The occurrence of nonreciprocal exchanges between y or sc and the centromere results in the formation of heteromorphic dyads, one being homozygous for the yellow mutant and for the distal extra heterochromatin, and the other for scute and lacking the extra heterochromatin. The effect of nonrandomness is apparent in the marked deficiency of homozygous yellow versus homozygous scute females in all three lines.

The results are as follows. Line 1 yielded 1194 non-yellow, non-scute females; 219 yellow and 616 scute females. Line 5 yielded 1148 non-yellow, non-scute females; 199 yellow and 531 scute females. Line 6 produced 898 non-yellow, non-scute females; 134 yellow females, and 403 scute females. These offspring resulted from the cross of the various attached-X females to y B (YSX.YL) males.

The c values or coefficients of nonrandomness, as determined by the ratio of scute females divided by the value of the sum of the scute and yellow females, for lines 1, 5, and 6 were .74, .73, and .75 respectively.

To determine the viability effect of the Y<sup>S</sup> heterochromatin in homozygous state, crosses were made of y cv v.Y<sup>S</sup>/y sc<sup>4</sup> B females to y cv v.Y<sup>S</sup> males and y cv v f/y sc<sup>4</sup> B females with y cv v f males. In this instance the extra heterochromatin was located proximally. The viability effects of the Y<sup>S</sup> arm in homozygous state were too small to account for the deviations which exist between the scute (short-armed) and yellow (long-armed) attached X's. The hypothesis of nonrandom disjunction of heteromorphic dyads accounts for the discrepancies noted in the various classes of attached X's.

Wette, Reimut. Production of melanogaster phenocopies with Nipagin M.

D. melanogaster were raised in a culture medium (synthetic medium after Pearl, DIS-6, containing about 15% killed fresh bakers' yeast but no live yeast added after cooling)

to which 0.75% Nipagin M dissolved in absolute ethanol had been added and homogeneously distributed before setting. The experimentals emerged 1-2 days later than the controls (0.075% Nipagin) and showed 10% phenocopies of the abnormal-abdomen type vs. 1.6% in the controls (Table 1). It is interesting to note that the sex ratio in the phenocopies is significantly increased (Table 2), whereas the over-all sex ratio does not differ from the hypotheti-

cal 1:1 nor from the controls. The probable cause of this phenomenon will not be discussed here.

Table 1

	Effect		
	No	Yes	Total
Experimental	1286	150	1436
Control	996	16	1012

Table 2

	Experimental			Control		
	No	Yes	Total	No	Yes	Total
Females	672	42	714	485	4	489
Males	614	108	722	511	12	523

Chi-square = 30.6 P << 0.001

Chi-square-Yates = 2.66 P ≥ 0.1

Widmer, E. A. An investigation of the mutagenic effect of 1-naphthalene acetic acid on D. melanogaster, using the aerosol technique.

indication of any positive mutagenic effect, but confirmation of these results is necessary.

Young, F. N., and Meyer, H. U. Another case of hymenopterous parasites attacking Drosophila in culture.

Muesebeck of the Entomology Research Branch (USDA) as Pachycrepoideus dubius Ashm. (Pteromalidae), which attacks a number of species of flies. Parasitization of pupae was high, approaching 80% of pupae. No reinestation has been noted.

Zimmering, S. Nonrandom disjunction in an unequal-armed translocation.

T(2;3)bwV4, which involves an exchange of almost all of 3L and the tip of 2R. The chromosomes in the translocation heterozygote may be described in terms of centromeres and associated arms: Let 2C represent the centromere and the right arm, respectively, of the normal second chromosome, 3D the centromere and left arm, respectively, of the normal third chromosome, and 2'D' and 3'C' those of the respective translocated chromosomes. In the absence of an exchange in the

The apparatus used was similar to the one described by Demerec (1948) in his work, and consisted of an air pump, electric timer, glass nebulizer, tubing, and treatment bottles. The chemical was dissolved in 95% ethyl alcohol. The results received gave no indication of any positive mutagenic effect, but confirmation of these results is necessary.

During the late spring of 1954, a culture of D. melanogaster in the Genetics Laboratory at Indiana University was observed to be infested with a small, ant-like wasp. The parasites were identified by Mr. C. F. W.

For information about the possible role of nonrandom disjunction as related to segregation in unequal-armed translocations (Novitski, 1951) a study was made of

interstitial region, that is, 2R, the chromosomes undergo either alternate or adjacent I disjunction, other types being rare (Glass, 1933). From alternate disjunction, then, gametes carrying 2C + 3D and 2'D' + 3'C' are formed, and if fertilized by a sperm of the proper constitution, will give rise to orthoploids identified as coming from alternate disjunction. Gametes of the constitution 2C + 3'C' and 2'D' + 3D are formed after adjacent I disjunction, and when complemented by the appropriate genotype from the male will result in aneuploid I individuals identified as coming from adjacent I disjunction. As a consequence of an exchange in the interstitial region, the above relationships may change. A single exchange in this region gives rise to asymmetric dyads of the types 2C.2D' and 2'C.2D', where D' is the longer and C the shorter of the two chromatids. On alternate disjunction, the crossover chromatids are now recovered as aneuploid I types, and those coming from adjacent I disjunction as orthoploids. Now, if, after the exchange, the longer of the two chromatids were, for some reason, at a relative disadvantage insofar as its chances for inclusion in the egg nucleus were concerned (as shown by Novitski for similar situations), then fewer gametes carrying the longer than the shorter chromatid would be produced. Hence one would expect a deficiency of individuals receiving 2'D' from the female as compared with those receiving 2C'. It is clear, therefore, that if the interstitial region were unmarked, those crossover types coming from alternate disjunction would be grouped with the general class identified as coming from adjacent I disjunction, and vice versa.

In earlier works (Glass, 1935) an approximate 50% discrepancy in the recovery of complementary aneuploid I classes was reported. Events in the interstitial region were not followed in these experiments, so that the results may be described with respect to combinations of centromeres recovered: in these cases, 2+ 3' from the female was recovered about twice as frequently as 2' + 3. This phenomenon was explained as resulting from the elimination of the latter type of individuals as a consequence of homozygosis for a postulated lethal in D and/or D', although, as was mentioned by the author, the expected frequency of homozygosis would be sufficient to account for something less than half of the observed deficiency. Other experiments ruled out conclusively that differential viability was responsible for the anomalous results.

On the basis of nonrandom disjunction (1) no discrepancy would be expected between complementary aneuploid I individuals which come from noncrossover tetrads, because of the absence of the asymmetric dyad, but (2) a discrepancy would appear in the relative frequencies of the complementary crossover classes as a result of more frequent production of gametes carrying the shorter chromatid than of those with the longer.

Tests were made in which crossing over was studied (1) in the interstitial region, (2) simultaneously in the interstitial region and in 3L, in which ve, h, and th were carried on the normal third chromosome, and (3) simultaneously in the interstitial region and in 3L, in which this arm of the translocated chromosome, 2'D', was replaced with a normal arm carrying ve, h, and th.

The results of these experiments showed the following: (1) that there was no difference in the degree of discrepancy between complementary aneuploid I classes as measured in females in which 3L of the translocated chromosome was or was not replaced with a normal homologue; (2) that the presence of a homozygous lethal was sufficient to account for less than half of the total deficiency found earlier; (3) that nonrandom disjunction operated after an exchange in the interstitial region such that 2D' + 3D was recovered about

half as frequently as 2'C + 3'C'; (4) that there was no difference in the relative frequencies of complementary noncrossover aneuploid I classes; and (5) that there was no discrepancy comparable in magnitude to that found earlier. Possibly related to (5) is the fact that the frequency of exchange in the interstitial region was surprisingly low, about 12%-15% in a region approximately 50 genetic units in length.

The c values (degree of nonrandomness) for complementary crossover classes were found to be as follows (taken from three experiments in which a reasonably large number of flies was scored): for those followed by alternate disjunction, .74, .73, and .75; for those followed by adjacent I disjunction from the corresponding experiments, .70, .69, and .65.

## TECHNICAL NOTES

Burdick, A. B. New medium of reproducible quality, stable at room temperature.

The following medium is the result of 3800 medium trials and has been in use in this laboratory since January 18, 1954. It has several advantages: (1) after pouring in

clean, unsterilized bottles it can be stored at room temperature for as long as a month without contamination or deterioration; (2) no perishable materials are used in its preparation; (3) inexperienced people are able to prepare and use it without difficulty; (4) it is of uniform quality from batch to batch; (5) yeast growth does not "run away" with a culture if larval development is slow; (6) mold growth is prevented and growth of wild yeast and bacteria inhibited until the cultures are in the second generation.

Water	1000
Agar*	18-22
Corn meal	60
Dry yeast (dead)	10
Acid mix**	50
Propionic acid***	5
Methyl parasept*** (12.5% in ETOH)	12

\* Adjust agar to climate: 18 parts in dry winter months, as much as 22 parts in humid months of summer.

\*\* Acid mix should be made up in advance and kept on shelf, as follows:

Sugar (sucrose)	300
NaNO <sub>3</sub>	30
K <sub>2</sub> HPO <sub>4</sub>	10
MgSO <sub>4</sub>	5
KCl	5
FeSO <sub>4</sub>	0.1

\*\*\* Propionic acid and methyl parasept are mold and bacteria inhibitors. If bacteria begin to build up in cultures, alternate periodically by substituting

NC1 (3 Normal)	12
Dowicide A (14.5% in ETOH)	10

for the propionic acid and methyl parasept. Methyl parasept may be obtained from the Heyden Chemical Corp., 393 Seventh Ave., New York 1, N.Y., Dowicide A from Dow Chemical Co., Midland, Mich.

Boil and stir water, agar, corn meal mixture until agar is dissolved; add yeast and acid mix and boil 5 minutes. Remove from fire, allow to cool slightly, and stir in propionic acid and methyl parasept (10 parts of 50% benzyl benzoate for mite control may be added at this time if necessary). Pour one inch deep in clean bottles.

Lewis, Herman W. Method for making glass micro-needle points, and method for holding larvae in transplantation experiments.

company, which can be used for this purpose. I use a portable dental drill

I have found it easy and convenient to grind down the point of glass micro-needles used in transplantation work with a small diamond-dusted dental wheel. There are various types of diamond-dusted discs and wheels, obtainable from any dental supply

for rotating the diamond-dusted wheel, and make the point while viewing it through a dissecting microscope.

To hold the etherized host larvae firmly in place during transplantation I have found it convenient to place them on a glass plate to which is attached some Scotch tape, adhesive side up. The larvae adhere to the Scotch tape very tenaciously during the transplantation procedure and can readily be removed afterward by placing a drop of water on the tape and floating the larvae off.

Lewis, Herman W. Rapid method of preparing and dispensing cornmeal-agar medium.

We have found it efficient to combine, by the use of a single piece of equipment, the method of preparing medium and the method of dispensing medium followed in two different laboratories. M. M. Green (DIS-25) described a rapid method of preparing medium, using a pressure cooker. R. P. Levine (personal communication) dispenses prepared medium into vials using an Army field autoclave as a sealed container, adapted so that compressed air can be introduced into the chamber to force hot medium through a tube connected to a controlling Morrison valve. We have further adapted the Army field autoclave so that the medium can be prepared in it under steam pressure, as described by Green, and then dispensed from it with air pressure as done in Levine's laboratory.

A fitting has been placed in the lid of a 25-quart-capacity Army field autoclave, type AI-5, to which is connected on the under side of the lid a copper tube, inner diameter 6 mm, which extends to the bottom of the inner chamber in which the food is prepared. On the upper side of the lid, a Superior vacuum valve is used to connect the fitting to a length of pressure tubing which in turn is connected to a Morrison valve. The Morrison valve has threaded into it a tapered nozzle to prevent dripping of the hot medium when the valve is closed. This entire connection is used to direct the medium from inside the autoclave to the vials or bottles to be filled. A second fitting placed in the lid of the autoclave is used to connect the inner chamber of the autoclave with the compressed air source by means of rubber tubing. The rubber tubing is joined to the fitting in the lid of the autoclave with a Lunkenheimer pressure valve. When the medium is cooking the Superior vacuum valve and the Lunkenheimer pressure valve are closed and the medium is cooked under steam pressure. When the medium is to be dispensed into the bottles or vials, the steam pressure is released through a safety valve in the lid of the autoclave and is replaced with air pressure by opening the pressure valve. The vacuum valve is then opened and the medium is forced through the tubing and valves under nine pounds of air pressure. To clean the system, hot water is forced through it under air pressure. The modifications of the field autoclave described above were designed and made by W. G. Strovink and J. W. Dunnell of the M.I.T. Biology Department Shop.

Sang, James H. Wet ashing of Drosophila.

Methods of dry or wet ashing of *Drosophila* are generally unsatisfactory and cumbersome, and do not usually give a reliable quantitative yield. The following modification of Middleton and Stuckey's technique has none of these defects when used for estimating the iron content of pupae, and would presumably prove as satisfactory for other metals.

100 mg of clean pupae are placed in an 8" x 1" Pyrex tube, moistened with 0.2 ml reagent-grade concentrated nitric, and "charred" by standing the tube on

a metal hot plate running at 320° C. After cooling, a further 0.1 ml of nitric is added, the top of the tube covered with a watch glass, and the digestion continued by heating to dryness and for a further 15 minutes. This process may have to be repeated if the ash has not then been reduced to homogeneous light brown powder. Ashing is completed by adding 0.1 ml of fuming nitric to the cooled powder and reheating the covered tube on the hot plate. The final creamy white residue can be taken up in solution by any of the standard methods (e.g., with warm dilute sulphuric). Glass chips should be used to prevent splattering, and a blank run to allow for contamination of the reagents.

Reference: Middleton, G., and Stuckey, R. E. 1954 The preparation of biological material for the determination of trace metals. II. A method for the destruction of organic matter in biological material. The Analyst 79: 138-42.

Schuellein, R. J.

Identification of D. persimilis larval instars.

In working out the rate of life cycle for D. persimilis much time was spent working out the features which distinguish the instars. It is useful to compare them with

those of D. melanogaster, a well-known form: first instar, two small mandibular hooks each with three teeth of identical height (melanogaster has one tooth per hook); second instar, larger mandibular hook with 4-5 large teeth, posterior tooth making an angle of 20° with anterior tooth (melanogaster has 2-3 teeth per hook); third instar, very large hook with 17-20 small teeth (melanogaster has 9-12 teeth per hook). For rapid identification under the dissecting scope: first-instar mandibular hooks appear as two small gray dots deeply imbedded in the larval envelope; second-instar are strikingly larger and appear shiny black, and the two mid-dorsal tracheae are clearly visible; third-instar have such massive hooks they are easily distinguished. The posterior-dorsal spiracles appear bright orange in the third instar whereas they are pale lemon in the second instar. This is a distinct advantage in identification when the larva is completely submerged in the agar medium with only spiracles in view.

Schuellein, R. J. Nutrient-agar culture medium for study of development of larva.

The following medium was used in the study of developmental rates from egg to eclosion in D. persimilis:

Nutrient agar	15 g
Dextrose	10 g
Bakers' yeast	11.5 g suspended in 45 cc distilled water (added to agar after autoclaving, before gelation)
H <sub>2</sub> O (distilled)	450 cc
Moldex	4 cc of 10% alcohol solution

Enough medium is poured to cover the bottom of a Petri dish. Its translucent quality has the advantage over other media such that larvae are easily identified at all stages of development. Too much medium in a dish permits the larvae to burrow to a depth which makes it difficult to distinguish instars. A molasses-agar medium supports larvae well enough, but the dark color makes observation difficult. The agar surface is dried with sterile paper toweling before eggs are planted, since excess moisture alters the time of first-instar development. As the larvae approach third instar, a small

rectangular piece of sterile paper toweling is placed on the medium surface to provide a site for pupation.

#### TEACHING NOTES

Burdick, A. B. Trihybrid with duplicate independent factors.

bright vermilion color. We refer to this stock as "Bright" and use it in the student laboratory as an unknown mutant type.

If a virgin Bright (i.e., *v*; *cn*; *st*) female is mated with a wild-type male, the *F*<sub>1</sub> males are Bright and the *F*<sub>1</sub> females are wild-type. This leads to the tentative conclusion that Bright is a sex-linked recessive. However, when an *F*<sub>2</sub> is produced from *F*<sub>1</sub> x *F*<sub>1</sub> mating it is in a ratio of about 2.5 Bright to 1 wild-type; or when an *F*<sub>1</sub> female is testcrossed to a Bright male parent, the ratio is 7 Bright to 1 wild-type. The Bright trait, which in *F*<sub>1</sub> looked like a sex-linked recessive, now looks somewhat like a dominant.

On the basis of the above data the student may conclude either (1) three duplicate independent genes, one sex-linked, or (2) two duplicate sex-linked genes with about 26% recombination. Test matings of *F*<sub>2</sub> Bright types with Bright parents are interesting if time permits. They yield 1:1, 3:1, and 7:1 ratios and indicate that conclusion (1) is correct.

Burdick, A. B. Effect of environment on segregation results.

Results may vary depending on the temperature used and the ability of the student to detect an "almost normal" *vg* wing. However, the experiment provides an impressive illustration of gene-environment interaction and, used late in the semester, moderates a student's faith in genetic ratios as such.

<u>Phenotype</u>	<u>At 70° F</u>	<u>At 80° F</u>
<i>vg</i>	50	4
+	46	46
<i>c</i>	4	50

Results may vary depending on the temperature used and the ability of the student to detect an "almost normal" *vg* wing. However, the experiment provides an impressive illustration of gene-environment interaction and, used late in the semester, moderates a student's faith in genetic ratios as such.

Burdick, Sally Ann.  
Student stocks, hybrids,  
and supplies.

The need for a commercial source of *Drosophila* stocks and supplies has long been evident. Requests to established laboratories from high schools and small colleges have been, many times, too numerous to accommodate. In addition, the need for advice on suggested crosses and technique, for supplies, and for segregating populations makes this a rather specialized area of *Drosophila* technology.

Two years ago, with the help of Dr. S. A. Rifenburgh and my husband, I set up a small laboratory in my basement. My purpose was, and still is, to make available to schools some of the wonderful teaching material to be found in *Drosophila*. I have worked with people teaching at all levels, from elementary school to postgraduate university, although the majority of my requests have come from college general biology and genetics teachers.

My stock list includes over 40 stocks and I can supply the necessary equipment, media, and instruction so that working with *Drosophila* does not become a complicated, technical chore. One feature of the service that has been very popular is the segregating population. I make up  $F_2$ 's and back-crosses and ship them in half-pint cream bottles so that they begin hatching about the time of arrival. In this way, the teacher is not bothered with the problem of media-making, rearing, crossing, and so forth, yet can provide living, segregating material for laboratory work.

I charge for the stocks and supplies, but the advice is free. I have undertaken more comprehensive consultation involving stock maintenance for a fee, but I am most interested in showing people how easy and how instructive it can be to teach with *Drosophila*.

#### NOMENCLATURE

Preservation of the name  
Drosophila immigrans  
 Sturtevant.

The fruit fly which was named *D. immigrans* by Sturtevant in 1921 has become a familiar species in genetical research. For more than 30 years no other name than *immigrans*

has been used for this species. It is the "type species" of an important subdivision of the genus *Drosophila*, "the *immigrans* group of species." It now appears that the name *immigrans* is threatened by priority. In 1952 Harrison suggested, in a study of the New Zealand Drosophilidae (Trans. Proc. Royal Society New Zealand, vol. 79, pp. 514-515) that *D. brouni* Hutton (1901, Trans. New Zealand Institute, vol. 33, p. 91) was an older name for *immigrans*, and cited evidence supporting the identity. Up to that time the name *D. brouni* had been considered unidentifiable. In order to preserve the well established name *immigrans* an application was made to the International Commission of Zoological Nomenclature, signed by Ernst Mayr, J. T. Patterson, Marshall P. Wheeler, and Warren P. Spencer, to use its Plenary Powers to suppress *D. brouni* in favor of *immigrans* (Bull. Zool. Nomencl., vol. 9, pt. 6, pp. 161-162). In this application it was pointed out that the type of *brouni* is a female and that it is sufficiently difficult to identify females in the *immigrans* group to make it questionable that the identity of *brouni* and *immigrans* is established unequivocally. This doubt has been challenged by Dr. Harrison in a letter to the Commission. Dr. Basden of Edinburgh, on the other hand, has suggested that no action by the Commission was necessary as long as the identity was not clearly established.

Regardless of the nomenclatural and taxonomic controversy implied by these letters it is evident that the vast majority of geneticists would deplore a shift of names for such a well known species. In view of the fact that the action of the International Commission is greatly influenced by advice received from working zoologists, all readers of DIS are urged to write to the Secretary of the International Commission in support of the application made by Mayr,

Patterson, Wheeler, and Spencer, citing the reference number Z.N.(S.)711. Letters should be addressed as follows: The Secretary, International Commission on Zoological Nomenclature, 28 Park Village East, Regent's Park, London, N.W.1, England. It might be added that Dr. Harrison himself has endorsed the endeavors to preserve the well known name Drosophila immigrans.---(Editor)

Burdick, A. B. CMI stocks. Stocks with dominant markers and inversions in all major chromosomes, referred to as "MI" stocks by Muller (DIS-16: 65), are called CMI or complete marked inversion stocks in our laboratory. We list three such stocks now, H-32, H-34, and H-36, and have several others in various stages of development. Such stocks must be (1) viable in "pure" culture, (2) capable of producing at least one type of genome that will effectively prevent crossing over throughout, and (3) maintainable without selection. Condition (2) is not always met, but the designation CMI indicates that the stock was put together with this condition foremost in mind and that the most effective crossover-suppressor genome from this stock has been tested for crossing over.

#### MATERIALS REQUESTED OR AVAILABLE

Bloomington, Indiana. Drosophila workers at Indiana University extend permission to cite any of the material which they have had or will have in any issue of DIS except where, in specific instances, a contrary statement is made.

H. Gloor (Department of Genetics, Rijksuniversiteit te Leiden, Leiden, Netherlands) would greatly appreciate obtaining any sort of mutant types (chromosomal aberrations, dominants, recessives) of D. hydei, for the purpose of locating and marking two lethals. Stocks already available to him are: vermillion, peach, and ebony.

Bruce Wallace (Cold Spring Harbor, New York) would like samples from several populations of D. melanogaster inhabiting small oceanic islands. Samples of about 50 nonvirgin females collected in vineyards, fruit orchards, and so forth, several miles from the laboratory would be appreciated.

UNESCO Symposium Proceedings. On August 20-23, 1953, a Symposium on Genetics of Population Structure was held in Pavia, Italy, Istituto di Genetica, under the auspices of UNESCO. The Proceedings of this Symposium have just been published and contain papers by: Clayton, Morris and Robertson, Falconer, Scossiroli, Wallace, Dobzhansky, Lerner, Clausen, Lewis, Buzzati-Traverso, and Haldane. Copies of the proceedings are available from Libreria Internazionale Garzanti, Palazzo Universitario, Pavia, Italy.

Katherine Armstrong, Mount Holyoke B.A., 1954, is now working on the problem of abnormal abdomen in *Drosophila* under the supervision of Dr. F. H. Sobels at the Genetics Institute in Utrecht, Netherlands. Miss Armstrong is the recipient of a Fulbright grant. (Reported by Dr. Alice L. Bull, Mount Holyoke College.)

Jack Bennett, at the University of Oklahoma, is doing some wild collecting in the Norman area and intends to study a part of the *Drosophila* population there. He will consider requests for samples of certain species. So far he has collected and tentatively identified the following: *melanogaster*, *bifurca*, some *affinis* group species, *macrospina*, *melanissima*, *micromelanica*, *robusta*, *busckii*, *carbonaria*, *duncani*, *pseudomelanica*, *putrida*, *ritae*, *testacea*, and *victoria*. Since the past summer was very dry, many of the above-listed species were found only once.

Sally Ann Burdick, who in the past has assisted in the laboratories of J. W. Gowen and Curt Stern, now has a *Drosophila* supply service for high schools and colleges where stocks are needed in student laboratories. (See Teaching Notes.) Her address is 614 Evergreen, West Lafayette, Indiana.

Frank C. Erk, Johns Hopkins Ph.D., 1952, and now Professor of Biology at Washington College, Chestertown, Maryland, is spending the present academic year at the University of Chicago on one of the internships offered by the Joint Program for Internships in General Education (Chicago, Columbia, Harvard, Yale). It is the first time a biologist has been awarded one of these internships. He is continuing his work on the fate of chromosome aberrations in experimental populations of *Drosophila*, and will return to Washington College next year.---(Reported by Dr. Bentley Glass.)

R. Milani will be in Rome at the Istituto Superiore di Sanità till October, 1955, working on house-fly genetics. After this time he is expected to resume his place in the Zoology Department of Pavia University.

Henry L. Plaine, Johns Hopkins Ph.D., 1954, has been appointed Assistant Professor of Genetics in the Department of Zoology and Entomology of Ohio State University. He will continue work on the chemical agents that affect the suppressor-erupt and suppressor-tumor systems, and particularly on the action of cysteine and related compounds as inhibitors of the effects of X-rays and of tryptophane on these gene-controlled systems.---(Reported by Dr. Glass.)

Maxwell E. Power, well known for his work on the neurology of *Drosophila*, was killed in an automobile accident in Iraq on the night of March 5, 1954. Professor of Biology at Kenyon College, Gambier, Ohio, Power was spending the year as a Fulbright Scholar teaching at Queen Aliyah College in Bagdad. Before going abroad he had been engaged on a study of the larval nervous system and the changes incident to metamorphosis to the adult condition. This work was not completed for publication, and the preparations which he had made for it as well as other preparations of adult nervous systems have been deposited as the nucleus of a permanent collection of *Drosophila* materials at the Osborn Zoological Laboratory, Yale University, New Haven, Connecticut, where his investigations were originally undertaken. The slides will be available for study at Yale by qualified investigators.---(D. F. Poulson)

Calcutta, India. The Department of Zoology, University of Calcutta, 35,

## RESEARCH NOTES

Abrahamson, S., and Herskowitz, I. H. The effect of X-ray intensity and dose on egg mortality following irradiation of female *Drosophila*.

(1955) was carried out to study egg mortality, as determined by the failure of eggs to hatch into larvae. The results prove there is a fraction of egg mortality which is intensity-dependent, the behavior of which parallels the effect of intensity on half-translocation frequency in all the respects tested: (1) Intensity effects (lowered frequency at lower intensity) were obtained for both egg mortality and half-translocations with similar intensity changes and over similar dose ranges. (2) The intensity effect for both decreased in eggs laid a number of days after treatment as compared with eggs laid earlier (although this decrease occurred in an earlier day in the case of half-translocations, for undetermined reasons). (3) The intensity effect for both was greater at lower doses delivered at lower intensities, when the contrasted durations of exposure were the same at two different doses. (4) In experiments performed in the same way, in which the intervals between successive intensely administered irradiations were varied but the total dose delivered remained constant, the amount of change was consistent for both types of effect: that is, whereas the treatments that were 30 minutes long (inclusive of intervals) gave results which were not statistically different from more condensed treatments, the frequencies obtained from two other treatments, which were more protracted, were significantly lower. With different X-ray doses a similar change in the frequency of half-translocation (Herskowitz, Proc. Nat. Acad. Sci. 40: 576-585, 1954) and of intensity-dependent egg mortality was also found. (5) Both frequencies increased with dose at a rate greater than expected on the basis of a one-event hypothesis of their origin. All this comprises evidence that the intensity-dependent component of egg mortality, which under certain conditions of irradiation can result in the death of at least 22.5% of all eggs oviposited, is largely the result of multiple X-ray-induced events. The hypothesis is suggested that the intensity-dependent fraction of egg mortality represents dominant lethal mutations which have their basis in two or more chromosome breaks in the maternal chromosomes. (This work was supported by a grant to Dr. H. J. Muller and associates from the Atomic Energy Commission, Contract AT (11-1)-195.)

Baker, William K. On the structure of the  $sc^8.Y:bw^+$  chromosome of *D. melanogaster*.

with  $Y^S$ . In conjunction with radiation experiments on the loss of the  $X^c$  chromosome, further evidence on the structure of the  $sc^8.Y:bw^+$  has been obtained.  $X^c, y/sc^8.Y:bw^+$ ;  $bw$  males were X-irradiated with doses varying from 450 to 1800 r and mated to  $y\ v\ bb/Y^cL$ ;  $bw$  females.  $F_1$  females whose Y chromosome had lost either or both of the markers,  $y^+$  or  $bw^+$ , were appropriately crossed to determine if the  $Y^S$  or  $Y^L$  fertility factors were present. The  $F_1$  females were not checked for bobbed unless  $y^+$  or  $bw^+$  was lost.

The types and frequencies of aberrant Y chromosomes recovered, out of a total of 42,370  $F_1$  flies, are presented in table 1.

A series of experiments, similar to those performed to study the effect of X-ray intensity and dose on the frequency of half-translocations induced in oocytes (Herskowitz and Abrahamson, Rec. Genet. Soc. Amer. 24; and Genetics 40: 574-575,

1955) was carried out to study egg mortality, as determined by the failure of eggs to hatch into larvae. The results prove there is a fraction of egg mortality which is intensity-dependent, the behavior of which parallels the effect of intensity on half-translocation frequency in all the respects tested: (1) Intensity effects (lowered frequency at lower intensity) were obtained for both egg mortality and half-translocations with similar intensity changes and over similar dose ranges. (2) The intensity effect for both decreased in eggs laid a number of days after treatment as compared with eggs laid earlier (although this decrease occurred in an earlier day in the case of half-translocations, for undetermined reasons). (3) The intensity effect for both was greater at lower doses delivered at lower intensities, when the contrasted durations of exposure were the same at two different doses. (4) In experiments performed in the same way, in which the intervals between successive intensely administered irradiations were varied but the total dose delivered remained constant, the amount of change was consistent for both types of effect: that is, whereas the treatments that were 30 minutes long (inclusive of intervals) gave results which were not statistically different from more condensed treatments, the frequencies obtained from two other treatments, which were more protracted, were significantly lower. With different X-ray doses a similar change in the frequency of half-translocation (Herskowitz, Proc. Nat. Acad. Sci. 40: 576-585, 1954) and of intensity-dependent egg mortality was also found. (5) Both frequencies increased with dose at a rate greater than expected on the basis of a one-event hypothesis of their origin. All this comprises evidence that the intensity-dependent component of egg mortality, which under certain conditions of irradiation can result in the death of at least 22.5% of all eggs oviposited, is largely the result of multiple X-ray-induced events. The hypothesis is suggested that the intensity-dependent fraction of egg mortality represents dominant lethal mutations which have their basis in two or more chromosome breaks in the maternal chromosomes. (This work was supported by a grant to Dr. H. J. Muller and associates from the Atomic Energy Commission, Contract AT (11-1)-195.)

This chromosome was obtained by Cooper (DIS-26) as a result of crossing over between the  $sc^8.Y$  and the  $Y:bw^+$ . He suggested that the  $sc^8$  piece is associated

with  $Y^S$ . In conjunction with radiation experiments on the loss of the  $X^c$  chromosome, further evidence on the structure of the  $sc^8.Y:bw^+$  has been obtained.  $X^c, y/sc^8.Y:bw^+$ ;  $bw$  males were X-irradiated with doses varying from 450 to 1800 r and mated to  $y\ v\ bb/Y^cL$ ;  $bw$  females.  $F_1$  females whose Y chromosome had lost either or both of the markers,  $y^+$  or  $bw^+$ , were appropriately crossed to determine if the  $Y^S$  or  $Y^L$  fertility factors were present. The  $F_1$  females were not checked for bobbed unless  $y^+$  or  $bw^+$  was lost.

The types and frequencies of aberrant Y chromosomes recovered, out of a total of 42,370  $F_1$  flies, are presented in table 1.

Table 1

Phenotype of F <sub>1</sub> Female	Genotypic Designation	Y <sup>L</sup>	bw <sup>+</sup>	sfa	bb <sup>+</sup>	Y <sup>S</sup>	y <sup>+</sup>	Number Tested	Estimated Number
y v bb bw (429)*	1	-	-	?	-	-	-	271	412
	2	+	-	?	-	-	-	8	12
	3	-	-	?	-	+	-	3	5
y v bw (95)	4	-	-	+	+	-	-	58	89
	5	+	-	+	+	-	-	4	6
v bw (14)	6	-	-	+	+	+	+	13	13
	7	+	-	+	+	+	+	1	1
y v (22)	8	-	+	+	+	-	-	8	14
	9	-	+	+	+	+	-	4	6
	10	+	+	+	+	+	-	1	2
y v bb (9)	11	-	+	+	-	-	-	4	7
	12	+	+	+	-	-	-	1	2

\* Numbers in parentheses indicate the total number of individuals observed of that particular phenotype.

Loss of all markers, genotype 1, could be caused either by complete loss of the irradiated Y chromosome or by loss of X<sup>c</sup> in 3X:2A zygotes. Either of these losses accompanied by primary nondisjunction in the parental females or by nondisjunction in the X<sup>L</sup>/Y<sup>S</sup> males, used to test for fertility factors, would account for genotypes 2 and 3 respectively. Thus these three genotypes provide no information about the structure of the chromosome in question. A qualitative examination of the other 9 genotypes suggests that the order of genes given at the top of the table is correct. It is interesting to note that a cytological check was made of two of the four cases of genotype 9 and both showed a ring-Y chromosome.

Other gene orders cannot be excluded solely by these qualitative considerations. Another approach is to determine the order which would require not only the least total number of breaks but also the lowest frequency of multi-break events, since the doses given were relatively low. These data, which are calculated on the basis of the last column of table 1, are shown in table 2. No consideration is given to which side of the sfa bb<sup>+</sup> is located. Of the twelve possible orders, the three shown in this table have the lowest break frequency.

Table 2

Order	If Other Chromosomes Involved					If Only Y is Involved		
	Number of cases with					Number of cases with		
	1 brk.	2 brk.	3 brk.	4 brk.	Total	2 brk.	4 brk.	Total
Y <sup>L</sup> bw <sup>+</sup> (sfa-bb <sup>+</sup> ) Y <sup>S</sup> y <sup>+</sup>	17	28	6	0	91	45	6	114
Y <sup>L</sup> bw <sup>+</sup> (sfa-bb <sup>+</sup> ) y <sup>+</sup> Y <sup>S</sup>	15	24	6	6	105	36	15	132
Y <sup>L</sup> (sfa-bb <sup>+</sup> ) bw <sup>+</sup> Y <sup>S</sup> y <sup>+</sup>	10	28	13	0	105	39	12	126

These data confirm the suggestion that the order given in table 1 is probably correct.

This gene order leads to an interesting conclusion as to the origin of the  $sc^8.Y:bw^+$  chromosome. If the particular  $sc^8.Y$  which gave rise to this chromosome is like other  $sc^8.Y$ 's with the  $sc^8$  piece at or near the tip of  $Y^L$ , then pairing and exchange probably took place between  $y^+$  and the  $Y^L$  fertility factors on the  $sc^8.Y$  chromosome and a region distal to the  $Y^S$  fertility factors on the  $Y:bw^+$  chromosome.

Barigozzi, C., and Di Pasquale, A. Further investigations on pseudotumors of *Drosophila*.

Localization of *tu* genes has been carried out for stocks A2 and B3 of *D. melanogaster*. In both stocks, mainly the second chromosome is involved in determining pseudotumors (see DIS-28); therefore, only this chromosome has been investigated. Using as markers *b* on *vg*, the following results have been obtained: in A2 the region acting on pseudotumors is located  $\pm 20$  units to the left of *b*; in B3, about 20 units to the right of *vg*. No structural differences in chromosomes have been observed between the stocks. Some evidence has been found to support the view that several *tu* genes are involved; hence, the loci indicated above must be understood as regions, where *tu* genes are especially concentrated.

Besides this genic determination, pseudotumors are connected with cytoplasmic activity. This has been proved by two methods: (1) Transfer of *tu* chromosomes into cytoplasm from no-tumor stocks; pseudotumor incidence is lowered. (2) Transfer of non-*tu* chromosomes into *tu* cytoplasm; pseudotumors are produced. No information has been obtained so far on the relation between gene and cytoplasm activity.

Crosses among stocks have failed to give pseudotumors, except in one single case (C4), which behaves as dominant. Selection experiments have been carried on. Selection for presence (+) and absence (-) of pseudotumors gave a prompt response, characterized by a strong change in incidence. The non-selected stocks proved to be highly heterozygous. The response to selection was especially strong in a genotype in which only the second *tu* chromosome was present.

To summarize: different stocks differ greatly with regard to genic location, and all cases investigated so far have shown cytoplasmic activity, integrating, in an unknown way, the action exerted by special chromosome regions.

Basden, E. B. Abnormalities in nature.

A total of 1250 specimens (524 *D. obscura*, 617 *D. subobscura*, 109 *D. silvestris*) reared from wild bait were examined for obvious abnormalities, of which 44 or 3.5% (10 *obscura*, 27 *subobscura*, 7 *silvestris*) were found. It is hoped that the work will be intensified during 1956.

Basden, E. B. Conspecificity of *Parascaptomyza disticha* (Duda) and "Scaptomyza graminum" (auctt.).

Reciprocal crosses were carried out between Spanish, English, and Scottish stocks of *P. disticha* and Missouri (U.S.A.) stock of "S. graminum." All matings produced fertile  $F_1$  except the cross  $\delta$  Missouri x ♀ Spanish (Barcelona). This gave a fair number of  $F_1$ , which did not reproduce. It thus appears that a degree of speciation is evident in *P. disticha*.

It is hoped to continue the study with stocks from many countries (see "Materials Requested").

Basden, E. B. Irregular development in D. kuntzei  
Duda.

Adults of Stock 387 (England, Bucks, caught August, 1954) and Stock S (Switzerland, Bremgarten, caught 1948 or before) of D. kuntzei were put together 29 December, 1954, at 18° C. ♂ 387 F<sub>3</sub> x ♀ S (F<sub>9</sub> at this Institute) for 7 weeks gave no progeny. ♂ S F<sub>9</sub> gave fertile F<sub>1</sub> adults after 1 week's cohabitation.

The individual stocks behaved as follows: 387 F<sub>3</sub> (Dec. '54) - F<sub>9</sub> (June '55) gave plenty progeny adults. S F<sub>9</sub> (Dec. '54 - April '55) gave unhatched eggs, no progeny--the same adults being used throughout.

On 30 April '55 these S adults still alive (now 18 weeks old) were put with virgin 387 adults (1-2 days old). At 4 June the results were: (a) (10 ♂ 387 F<sub>6</sub> x 9 ♀ S F<sub>9</sub>) plenty unhatched eggs, no progeny. (b) (3 ♂ S F<sub>9</sub> x 10 ♀ 387 F<sub>6</sub>) plenty adult progeny, the first fertile eggs being laid 5-9 May.

From these results we gather: (1) The Swiss F<sub>9</sub> adults (em. Nov. '54) were productive but the F<sub>9</sub> (em. Dec. '54) were not. (2) Adults of the recently caught English stock remained productive throughout, (but see last paragraph below). (3) Crossing showed that Swiss males were productive even after 18-22 weeks but that Swiss females (F<sub>9</sub>) were unproductive. These females laid many eggs, which did not hatch after 4 weeks (? fertile ? diapause). (4) Since the Swiss females laid many eggs, it seems unlikely that they alone of the two sexes were in a reproductive diapause.

Another English stock (No. 392, Kent, 4♂ 3♀ caught Aug. '55) laid many eggs at 18° C, but none has hatched (22 Oct.). Dissection of one pair showed motile sperm (and parasitic worms) in the male, no sperm in the female; the female had laid eggs.

Bateman, A. J. Inheritance of egg hatchability.

It was found that the F<sub>1</sub> hybrid between the two inbred lines Brum 1 and Oregon had a very low percentage of nonhatching eggs--0.83% excluding the first sperm batches, which showed a slightly higher percentage. This probably corresponds to the spontaneous rate of dominant-lethal mutations. The reciprocal cross, however, had a nonhatching percentage of 35%. Further investigation disclosed some rather peculiar features in the heredity of hatchability in this material. (1) Cytoplasmic inheritance: This was partly responsible for the above reciprocal difference. (2) Maternal chromosomal genotype: The influence of the mother seemed to be polygenically determined in a straightforward way, showed heterosis in the F<sub>1</sub>, with segregation and intermediate values between F<sub>1</sub> and parents in back-crosses. (3) Paternal genotype: This seemed relatively trivial, but there was a strong interaction with the egg cytoplasm, hatchability being improved if the sperm entered cytoplasm from its own line. (4) Genotype of the fertilized egg: This was the least important factor of all! The highest hatchability was found in F<sub>2</sub> eggs. Though these might be expected to be the most unbalanced genetically, there was evidently nothing severe enough to impair hatchability. In contrast, F<sub>1</sub> eggs, expected to show most heterosis were among the worst for hatchability because of the incompatibility between sperm and cytoplasm.

Bateman, A. J. Relative biological efficiency of 4 MeV linear accelerator.

For this comparison with a 300 keV Resomax machine, the above-described high-hatchability material was used and the RBE was measured by comparison of the dominant-

lethal rates induced by 2000 r. It was expected that this would provide one of the most sensitive biological tests possible. Nevertheless, the error variation was sufficient to prevent a clear result.

In one series, 300 keV gave a dominant-lethal percentage of  $28.69 \pm 0.64$ , whereas 4 MeV gave  $21.79 \pm 0.70$ . The difference was highly significant and the estimate of RBE became  $75.95 \pm 3.0$ . (N.B. My error estimates are not *a priori* but are based on the error variation between dominant-lethal estimates for individual males.)

In the combined data, however, the results were not so tidy, the individual estimates of RBE being respectively 87.49, 75.96, 91.83, 82.86, 94.82, 75.07, and 109.10. The pooled RBE becomes 87.93 and the significance of its deviation from 100% is just below the 5% level. It is my personal conviction that, in this material, there is a real difference in the biological efficiency of 4 MeV and 0.3 MeV X-rays, to the disadvantage of the higher energy, but that it is unlikely to be greater than 20%.

Beardmore, J. A. Homeostasis in constant and fluctuating temperatures.

The homeostatic properties of inbred Oregon and Samarkand stocks and their F<sub>1</sub>'s have been compared in two environments, one a constant 25° C, the other having the

same mean (25°) but a diurnal fluctuation from 20° C at midnight to 30° C at midday. Counts of sternopleural bristles confirm Mather's observation that the F<sub>1</sub>'s are less asymmetrical than the parental types in the constant environment. In the fluctuating environment, however, the reverse is the case (interaction  $p = < 0.01$ ). An increase of asymmetry in the fluctuating compared with the constant environment occurs in both inbreds and F<sub>1</sub>'s but the increase is relatively and absolutely greater in the F<sub>1</sub>'s. Since asymmetry is a valid measure of homeostatic ability, the inbred stocks have buffering mechanisms superior to those of their F<sub>1</sub>'s in the fluctuating environment.

Preliminary tests of wild stocks in these environments indicate that shortly after initial capture they are less asymmetrical in the fluctuating than in the constant environment but that after some months in a constant-temperature room this adaptation to the fluctuating conditions disappears.

These results must affect our interpretation of the results obtained from testing wild chromosomes singly and in combinations in constant-temperature conditions.

Begg, Michael. Osmotic pressure of *Drosophila* larval hemolymph.

In the course of work on in vitro culture of *Drosophila* tissues, it became necessary to obtain a value for the osmotic pressure of larval hemolymph. This was accomplished

by using a modification of the thermoelectric vapor pressure method of Baldes. A value equivalent to aqueous 1.05% NaCl has been obtained, and this appears to be more or less constant for different larval stages. Sterile fragments of larval gut suspended in such a solution in hanging drops of phenyl-thiourea-treated hemolymph have continued to show spontaneous contractions for over 90 hours at  $24^\circ \pm 1^\circ$  C.

Bowman, J. T., and Jacobs, M. E.  
A completely penetrant melanotic tumor strain of D. melanogaster from a wild population.

obtained by selection. The penetrance of the tumor increased almost linearly from .46 to 1.0 in six generations of selection. Penetrance remained complete on the usual yeast-cornmeal-syrup-agar medium at 18°, 22°, 25°, and 30° C. Development of the tumors is marked, and they are readily visible in the adult. Individuals with from one to eight tumors have been noted. Tumors are usually in the abdomen (commonly ventrally, but varying widely in position) but are also sometimes found in the thorax and, rarely, in the head. Viability of the strain is good, and a genetical analysis is under way.

Brosseau, George E., Jr.  
Meiotic and somatic behavior of a ring-X fragment in D. melanogaster.

with the Y, occurs in about 60% of the cases; occasionally the fragment apparently induces nondisjunction of X and Y. Meiotic loss is more frequent when the fragment disjoins from the Y than from the X. Oogenesis: By means of the XY chromosome and various attached X's it was possible to determine behavior with no, one, and two Y's, and one and two fragments. With either no or two Y's, the fragment disjoins from the attached-X about 70% of the time; with one Y, the fragment segregates with the attached-X about 60% of the time. Meiotic loss is most apparent when two fragments are present. Differential viability: Females will tolerate two fragments with no apparent effect on viability; males will similarly tolerate one fragment, but two either are lethal or reduce viability considerably. Somatic events: Flies hemi- or homozygous for y and carrying the fragment showed mosaicism for y. Small spots of 1-2 bristles may be attributable to position effect; large spots--that is, half an abdomen or thorax--seem best explained as resulting from loss of fragment. Unlike  $X^{c2}$ , such losses are not influenced by prior aging of maternal parent.

Brosseau, George E., Jr.  
The influence of a small ring chromosome on the somatic stability of  $X^{c2}$  in D. melanogaster.

three days after mating by unaged mothers and those aged as virgins for 8 days at 25° C. (See table on p. 107.)

A comparison of the frequency of gynandromers in fragment- and non-fragment-bearing sisters shows that the entire increase over the control is in the fragment-bearing class. This increase is not significantly affected by prior aging, and the  $sn^3$  mosaic areas are smaller than those typical of eliminations induced by aging. Thus the fragment acts later in ontogeny and independently of the aging maternal effect.

This strain was isolated from a wild population collected on October 21, 1954, at Beaufort, N.C., and kept in mass culture. Tumored flies were found in the culture and a true-breeding strain was

obtained by selection. The penetrance of the tumor increased almost linearly from .46 to 1.0 in six generations of selection. Penetrance remained complete on the usual yeast-cornmeal-syrup-agar medium at 18°, 22°, 25°, and 30° C. Development of the tumors is marked, and they are readily visible in the adult. Individuals with from one to eight tumors have been noted. Tumors are usually in the abdomen (commonly ventrally, but varying widely in position) but are also sometimes found in the thorax and, rarely, in the head. Viability of the strain is good, and a genetical analysis is under way.

Del( $X^{c2}$ )53d (Brown, DIS-29), a free ring fragment about half the diameter of  $X^{c2}$  (salivaries show 1A-F), is unstable in mitosis and meiosis. Spermatogenesis: Disjunction from the X, and segregation

with the Y, occurs in about 60% of the cases; occasionally the fragment

apparently induces nondisjunction of X and Y. Meiotic loss is more frequent when the fragment disjoins from the Y than from the X. Oogenesis: By means of the XY chromosome and various attached X's it was possible to determine behavior with no, one, and two Y's, and one and two fragments. With either no or two Y's, the fragment disjoins from the attached-X about 70% of the time; with one Y, the fragment segregates with the attached-X about 60% of the time. Meiotic loss is most apparent when two fragments are present.

Differential viability: Females will tolerate two fragments with no apparent effect on viability; males will similarly tolerate one fragment, but two either are lethal or reduce viability considerably. Somatic events: Flies

hemi- or homozygous for y and carrying the fragment showed mosaicism for y. Small spots of 1-2 bristles may be attributable to position effect; large spots--that is, half an abdomen or thorax--seem best explained as resulting from loss of fragment. Unlike  $X^{c2}$ , such losses are not influenced by prior aging of maternal parent.

The somatic instability of the ring-X chromosome is greatly increased when  $del(X^{c2})53d$  is present. In the cross  $y\ w\ sn^3/del(X^{c2})$  x  $X^{c2}$  the following percentages of gynandromorphs were obtained from the eggs laid in the first

three days after mating by unaged mothers and those aged as virgins for 8

days

at 25° C. (See table on p. 107.)

Maternal genotype	Total flies	y w sn <sup>3</sup> /X <sup>c2</sup>			y w sn <sup>3</sup> /X <sup>c2</sup> /del(X <sup>c2</sup> )			Totals		
		No.	No.	%	No.	No.	%	No.	No.	%
<b>y w sn<sup>3</sup></b>										
Unaged	3055	1531	9	0.59	-	-	-	9	0.56	
Aged	5822	2830	46	1.62**	-	-	-	46	1.62**	
<b>y w sn<sup>3</sup>/del(X<sup>c2</sup>)</b>										
Unaged	2544	723.6 <sup>a</sup>	7 <sup>b</sup>	0.97	482.4 <sup>a</sup>	43 <sup>c</sup>	8.91	50	4.14	
Aged	4089	1228.2 <sup>a</sup>	29 <sup>b</sup>	2.36*	818.8 <sup>a</sup>	99 <sup>c</sup>	12.09	128	6.25*	

<sup>a</sup> Estimated on the basis of 40% recovery of the fragment in the female.

<sup>b</sup> y sn<sup>3</sup> in mosaic areas.

<sup>c</sup> sn<sup>3</sup> only in mosaic areas.

\* Significant at the 5% level.

\*\* Significant at the 1% level.

Brown, Wm. P., and Bell, A. E.  
Lethal and sterility analysis  
of a closed population of D. melanogaster plateaued for  
fecundity.

A closed population which had been selected for high fecundity on an individual and family basis for 47 generations was observed to show a lack of response to selection, or a plateau for the selected trait, after the seventh selected generation.

Rapid progress had been made in the first seven generations from the level of the initial population, which was made up from four wild-type laboratory stocks. The closed population continued to exhibit high phenotypic variability, and heritability estimates were obtained after many generations of selection. Selection differentials were apparent each generation. Of the many possible reasons to account for this plateauing, balanced lethal factors and sterility factors are the most obvious. In an effort to evaluate the significance of these factors, lines isogenic for chromosomes 1, 2, and 3 were obtained from the closed population by an out-cross technique (a modification of Muller's system for the construction of homozygous lines). An analyzer stock with the following genetic constitution was used in the technique: sc<sup>1</sup> B InS w<sup>a</sup> sc<sup>8</sup>; InS<sup>1</sup>, a<sup>1</sup> Cy sp<sup>2</sup>/Pm ds<sup>33K</sup>; Ubx<sup>130</sup> e<sup>S/C</sup> Sb; pol. The B, Cy, and Ubx chromosomes are the important recombination-prohibiting chromosomes.

Virgin females from the closed population were mated to analyzer-stock males. The progeny of this cross were analyzed for sex ratio since information as to sex-linked lethal genes could be obtained as the presence of any such genes would be revealed in a 2 female-to-1 male sex ratio. Of the 57 crosses analyzed for sex ratio only three, or 5.26%, gave a good fit to a 2 female-to-1 male sex ratio. It is felt that this frequency of sex-linked lethals is too low to account for plateauing for fecundity in the closed population.

One hundred and twenty-six genomes from the closed population were successfully carried through to the final mating producing homozygous individuals. Only three of the genome samples gave evidence of the presence of an autosomal lethal effect. This indicates a lethal frequency of about .05. The lethal effects appeared to be associated with the second chromosome. It is felt that the lethal frequency of .05 is of too small a magnitude to explain the plateauing for fecundity in the closed population.

The isolated isogenic lines provided a measure for the presence of

sterility factors in the closed population. A sterility gene, as a lethal gene, would be made homozygous in the isogenic progeny by the outcross technique, and the effects of it would be observed in the inability of isogenic lines to produce progeny. Nearly 100% of the isogenic lines produced progeny in the first generation after isolation. It is felt that sterility factors along with sex-linked and autosomal lethal factors, as mentioned above, are not responsible for the plateauing for fecundity in the closed population. Relative viability analysis of homozygous vs. heterozygous chromosome types is now being made in an attempt to determine the cause for plateauing for fecundity in the closed population.

Brunstrom, Ola. Maternal influence on variegation in the M-5 chromosome.

The  $y^+$  and  $ac^+$  genes in the Muller-5 chromosome ( $sc^{S1} InS B w^a sc^8$ ) show variegation with low penetrance. The variegation has been measured for the  $y^+$

gene by determining the frequency of animals with one or several yellow bristles on the thorax. Yellow females from different  $y$  stocks were mated with M-5 males, and the proportion of daughters showing variegation was determined. The degree of variegation is a condition of the  $y$  stock of the mother. By changing autosomes between two  $y$  stocks, which gave, respectively, 1% and 10.6% mosaics among the daughters, it was concluded that the maternal effect is modified by a gene in the third chromosome. This gene is dominant for the suppression of variegation. A chromosome 3 with the recessive gene for enhancing variegation in the offspring was brought into the M-5 stock. It was followed by an increase of mosaics in the M-5 stock for  $y$  as well as  $ac$ . The rate of  $y$  mosaics is higher in M-5 homozygotes than in the heterozygote M-5/ $y$ . See below.

Abbreviations:  $III^h$  = Chromosome III with the recessive gene giving high rate of mosaics in the offspring.  $III^l$  = chromosome III with the dominant gene giving low rate of mosaics in the offspring.

$y III^l \times M-5$	1.0% $y$ mosaics among the daughters
$y III^h \times M-5$	10.6% $y$ mosaics among the daughters
$M-5 III^h \times M-5$	(14.7% $y$ mosaics among the daughters (3.0% $y$ mosaics among the sons)
$\frac{y}{M-5} III^h \times M-5$	(9.8% $y$ mosaics among the $\frac{y}{M-5}$ daughters (16.2% $y$ mosaics among the M-5 daughters)

XO males from the M-5  $III^h$  stock show variegation in 89.0%, and XO males from the M-5  $III^l$  stock 52.2%.

Burdette, Walter J. Change in tumor incidence following irradiation.

Males of the  $tu vg bw$  strain were irradiated when three days of age in a single treatment consisting of 2000 roentgens. They were then mated to

nonirradiated females of the same strain, and flies in the F<sub>1</sub> generation were carefully examined. Those without tumors were mated to  $sc^{S1} B InS w^a sc^8 Cy/Pm$ , H/Sb males, and  $vg bw$  flies were checked for tumors in subsequent generations. Lethal mutation rate on the X chromosome was also determined and found to be 2.8% (208/7389) in the irradiated and 0.12% (7/5638) in the nonirradiated group. There were 74 flies (11 males and 63 females) without tumors among 52,985 counted in control studies, but offspring of all 62 followed in subsequent generations developed tumors in characteristic numbers.

In the irradiated group, 72,666 were examined and 105 did not have tumors. As in the control group, the number of males without tumors (22/36,491) was less than the number of females without them (83/36,175). However, in offspring of two of the 76 flies which were observed in subsequent generations the incidence of tumors has been consistently less than one-third that in the parent stock. Experiments are in progress to determine whether this is due to mutation of a modifier or reversal of an original tumor gene.

Carlson, E. A. The vortex and comma effects of the dumpy locus.

Studies of the pseudoalleles of the dumpy locus (Muller, Meyer, and Carlson, *Genetics* 40: 587) showed that at least two loci or regions are present in the dumpy series--oblique and vortex. It is also noteworthy (and has long been known) that various members of this series show much variation in the size and number of the vortices, the size and shape of the wings, and a number of other features. Only the vortex and, to a lesser extent, comma characters are considered here. The alleles best studied by the writer in these respects will be presented in order of intensity of the vortex character, as judged by their homozygotes when these survive, and by their compounds with dumpy. The following observations must so far be regarded as provisional since some of the differences seen may have been caused by modifying genes and/or environmental conditions, especially since these phenotypes are known to be so readily modifiable in such ways.

1, 2, 3) The obliques (which in order of their amount of wing shortening are  $dp^{o3}$  of Muller,  $dp^o$  of Bridges, and  $dp^{o2}$  of Laemmerts) do not manifest vortex when homozygous or even when compounded with the most extreme vortex, thoraxate, or truncate alleles.

4) Lopped of Meyer ( $dp^{T51b}$ ) when in compound with  $dp$  seldom shows vortices, and these rare cases usually show them on one side only; when compounded with  $dp^{V2}$  it never shows vortices; hence  $dp^{T51b}$  might be considered very mild in its vortex effect. No comma effect is ever expressed in the compounds.

5) The original vortex of Bridges ( $dp^V$ ) when homozygous does not show vortices unless an intensifier in the third chromosome (vo-3) is also present, and then shows them only weakly, but when compounded with  $dp$  it shows more pronounced vortices than does lopped. The comma effect is not present in the homozygote or compound with dumpy.

6) A different vortex effect, which probably lies between those of  $dp^V$  and  $dp^{V2}$  in average intensity, is found in the dumpy-warped of Schalet ( $dp^W$ ). In the form  $dp^W/dp$  the wings are usually asymmetrical in size. This is true also for the vortices which usually appear as one or two nodes on the side in which the shorter wing appears. No comma effect is seen in the compounds.

7) Vortex-2 of Mohr ( $dp^{V2}$ ) when homozygous shows from two to four vortices with little phenotypic overlapping of the wild type. It does not require a modifier. Marked vortices appear in its compound with  $dp$ . There is no comma effect in the homozygote or compound with  $dp$ .

8) Dumpy of Morgan ( $dp$ ) when homozygous shows a vortex effect in almost all the flies, with about 90% showing comma. Vortices are more typically of the volcano type than the pitted.

9) Dumpy-humpy-like of Edmondson ( $dp^h$ ) as a homozygote shows lumping

and comma effects like those of  $dp^{TC-2}/dp$ , but the microchaetae are not affected. Its compound with  $dp$ , while more marked in regard to vortices and commas than is homozygous  $dp$ , is less marked than the compounds of dumpy with the truncates.

10) Thoraxate of Bridges ( $dp^{tx}$ ) when in compound with  $dp$  shows pronounced vortices, either darkly pitted or erupted into mounds. The comma is not always present in  $dp^{tx}/dp$ .

11) Truncate of Schalet ( $dp^T-Sch$ ), judged by genetic evidence to be a deficiency, in the form  $dp^T-Sch/dp$  shows a vortex effect about equal to that of  $dp^{tx}/dp$ , but the volcanoes, very seldom more than two, are broader. Its comma effect is strong, but is without the "shelf" seen in  $dp^T$ .

12) Truncate of Morgan, 1923 ( $dp^T$ ) usually shows all four vortices affected when compounded with  $dp$ . The commas sharply define a projecting "shelf" at the anterior thorax.

13) Truncate of Carlson-55e ( $dp^{TC-1}$ ), the first obtained by this writer which may have arisen by crossing over between  $dp^{tx}/dp^{o2}$ , as a compound with  $dp$  shows even more extreme vortices and commas than  $dp^T/dp$ .

14) Thoraxate of Ives ( $dp^{txI}$ , but referred to as  $dp^T$  in the Bloomington stock list), which has always been included in a Cy inversion, shows a very strong vortex lumping and furrowed commas in  $dp^{txI} Cy/dp$ . No comma is present when compounded with  $dp^{v2}$  and the vortices are weaker in appearance, with pitting frequent.

15) Truncate of Carlson-55f ( $dp^{TC-2}$ ), derived from a  $dp^{o2}/dp^{tx}$  female, is severely lumped when compounded with  $dp$ ; furrowed commas produce a sharply raised "shelf." The microchaetae are perpendicular to the surface of the thorax.

16) Finally, umpy-lumpy of Carlson-55g ( $dp^{ly}$ ), which also arose from a  $dp^{o2}/dp^{tx}$  female, when compounded with  $dp$ , has huge vortices thrown forward into bulges resembling shoulder padding, and the comma effect is so extreme that the "shelf" seems to be a wedge or disk held by the "pads."

An atypical vortex character is the umpy-commma of Meyer ( $dp^{cm-2}$ ), described in a separate account in this issue) in which the homozygous fly shows no vortex but only the commas and "shelf." The vortex is expressed, however, in a number of its compounds with different dumpy alleles. Strange to say, the order of intensity of vortex shown by these compounds appears to be in radical disagreement with the above order, based on the intensity of vortex in homozygotes and in compounds with  $dp$ .

These observations on vortex and comma indicate the great variety of effects of the dumpy pseudoalleles, and the potentialities which they afford for analyzing the very obviously complex behavior of the genes concerned.

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Castiglioni, M. C. Phenogenetics of pseudotumors in *Drosophila*.

showing possible differences between larval hemolymph of tumorous and tumor-

Recent investigations on phenogenetics of pseudotumors in *D. melanogaster* have been directed chiefly towards

less stocks. A careful cytological examination permits us to classify the elements present in the normal hemolymph, with regard to their size and cytoplasmic structure, as follows: (1) Very small cells (4-6 microns): their cytoplasm is deeply basophilic and the nucleus not always evident. (2) Small cells (10-13 microns): the basophilic cytoplasm appears homogeneous. (3) Medium-sized cells (16-20 microns): they are spherical or ovoid in shape; the cytoplasm, which is moderately basophilic, contains numerous vacuoles and inclusions. (4) Large cells (24-28 microns): the cytoplasm is lightly basophilic and only a few vacuoles are present. (5) Crystalloid cells: many crystal-like inclusions are found in their strongly basophilic cytoplasm.

All the types of cells listed above are present also in the hemolymph of tumorous larvae at different ages. But the most interesting observation is a highly significant difference in the number of large cells: less than 1% in larvae of a tumorless stock, reaching the value of 30% in larvae of a stock with pseudotumors. These cells seem to be the essential elements in the formation of pseudotumors.

Four phases may be recognized in processes leading to the appearance of melanotic masses: (1) abnormal increase in number of the largest hemolymph cells; (2) aggregation of such cells; (3) initial deposition of melanin around the cells; (4) complete melanization, with no cytological structure now visible.

Chung, Y. J. Collection of wild Drosophila on Quelpart Island, Korea.

Collections of Drosophila were made in various localities on Quelpart Island by the writer and K. W. Kim in July and August, 1955. D. auraria, D. immigrans, D. melanogaster, D. suzukii, and D. virilis were collected in five localities--Chaeju, Shungsapo, Sugipo, Mosilpo, and Mr. Hanrah--with native-fruit-baited traps. D. melanogaster and D. virilis were found abundantly, the former outdoors, the latter indoors; and both seem to show a much wider distribution than other species.

Cooper, K. W. Suppression of wing speckling by Y chromosomes.

T(2;3)Arista (DIS-28: 74-75) has two associated dominant phenotypic effects which serve to mark the translocation: (1) a reduction of the lateral branches of the arista, and (2) the production of many small, transparent spots on the wing membranes by localized failures of hair formation. Classification is excellent for both characters. It is a noteworthy fact that supernumerary Y chromosomes have a pronounced effect on the expression of wing speckling, but not on aristal reduction. The speckling of the wings is completely suppressed (or very nearly so) in X2Y and X3Y males and in XXY and XX2Y females. Very likely the speckling is a consequence of one or more of the heterochromatic rearrangements of the translocation and, like many of the rearrangements that mottle the eye, is reverted to apparently normal (+) expression by extra Y chromosomes. In the presence of 2 Y chromosomes above normal for a genotype, the Arista phenotype no longer gives clean-cut classification, although it is assuredly not regularly suppressed. One extra Y chromosome seems to have little effect on its expression. T(2;3)Arista, then, may involve two different primary genetic changes; or speckling and aristal reduction may reflect different threshold responses to gene action by wing and antennal tissues. With care, it makes it possible to select XY, X2Y, X3Y, XX, XXY, and XX2Y from a large progeny of a single-pair mating.

Fahmy, O. G., and Fahmy,  
Myrtle J. Selective mutagenic action of the alkylating mutagens in D. melanogaster.

Gross differences in the mutagenic properties of chemical and radiation mutagens have long been recognized. Foremost among these is the low frequency of viable major chromosome rearrangements and the high frequency of small deficiencies induced by the chemical agents. A particular compound, *p*-N-di(chloroethyl)-phenylalanine, has been found to be extreme as to the low frequency of major rearrangements it induces. Compared to mutagenically equivalent doses of X-rays (as regards the sex-linked recessive lethal rate) the above amino acid mustard induces: one-twentieth the rate of viable translocations (between the second and third autosomes), one-fifteenth the rate of rearrangement recessive lethals, and one-tenth the rate of recoverable fragments.

Detailed analysis of the mutations induced by the alkylating compounds (mustards, epoxides, imines, and sulphonic esters) and their comparison with those induced by radiation, have now yielded decisive evidence as to the differential response of gene loci to the action of different mutagens. By the use of a few alkylating compounds it was possible to induce nearly 200 easily detectable (rank 1 and 2) "new" sex-linked recessive visibles. The phenotypic expressions and genetic positions of these visibles are completely different from those induced by other mutagens, especially X-radiation. Cytological analysis of a sample of these visibles showed that they are not associated with chromosome aberrations detectable in the salivary-gland chromosomes. It is clear, therefore, that the selectivity here discernible is for certain morphogenesis loci and not an expression of differential induction of chromosome-rearrangement mutations.

The differential action of the various mutagens on the gene complement has also been manifest in the variation of the ratio of visibles to lethals they induce in the same sample of treated chromosomes. The phenylalanine mustard was found to be especially effective in the induction of visibles, mutating 2 to 3 times as many visible loci relative to lethals as any other alkylating compound or X-radiation.

The distribution of the loci of recessive lethals along the X chromosome is the same for three representative alkylating mutagens and is significantly different from that for X-rays. A significant difference also occurs in the distribution of the  $F_1$  viable breaks along the X chromosome induced by an imine, tri(ethyleneimino)-triazine, as compared to X-radiation.

The above results constitute a fundamental divergence from the classical concept as to the manner of occurrence of mutations. They provide decisive evidence that the mutation process is by no means a random one, but is somehow dependent on the nature of the mutagen.

Fahmy, O. G., and Fahmy,  
Myrtle J. The cell stage during spermatogenesis and the yield of different mutations in D. melanogaster.

intervals and the mutation rate was scored separately in each brood. The mutation rate/brood pattern for the same genetic property showed a certain degree of variability in different experiments. The cause of variation has been traced to differences in the morphological status of the testis at the

The mutation yield of the various stages of spermatogenesis under the effect of 2:4:6-tri(ethyleneimino)-1:3:5-triazine has been investigated by the brood technique. The treated male was mated to a succession of virgin females at set

time of treatment, which depends on the speed of germ-line differentiation and cell damage resulting from the treatment itself. Another controlling factor is the way the male germ line is fractionated during the experiment, which varies with the brood interval. Variability between experiments could be reduced, sometimes even eliminated, by careful standardization of the brood technique: using males of the same age and average weight, rearing them under strictly regulated cultural conditions, and fractionating their progeny at the same fixed intervals.

The combined cytological and genetical investigation of the effect of the above imine on the morphology and mutagenic response of the treated testis enabled the following conclusion. Young males (30±5 hours) treated with up to  $3.5 \times 10^{-4}$  M of the compound, whose progeny was fractionated by repeated matings to fresh virgin females every 3 days, were found to ejaculate sperm derived from postmeiotic germ cells in four broods of 3 days each, that is, 12 days after treatment (Fahmy and Fahmy, 1954). Evidence was also available that, under the above conditions, spermatids with nuclei at the early condensation stage are called upon to supply sperm used between the 8th and 9th days after treatment, that is, towards the end of the third brood (Fahmy and Fahmy, 1955b). The various mutations studied give different brood patterns, which fall into three classes. Dominant lethals are a class of their own and their yield is highest among mature sperm (brood I), lowest among the latest spermatids (brood II), and intermediate for the earlier stages (broods III and IV). Minutes and chromosome fragments constitute the second class and their yield is highest among mature sperm and the latest spermatids (broods I and II) and falls sharply for the earlier stages (broods III and IV). The third class comprises the sex-linked recessive lethals and visibles and their yield peaks at the third brood. This has been shown to be due to the high sensitivity of the spermatids at the earliest stage of nuclear condensation (used towards the end of brood III) to the induction of intragenic mutations.

The sex-linked recessive lethals induced by the imine are associated with a high frequency of small deficiencies, amounting to 40% at some doses. The fact that in spite of this high frequency of deficiencies their pattern of yield still conforms with that of the recessive visibles (which are unassociated with chromosome aberrations) suggests that the imine deficiencies are a special type of intragenic change. This is in harmony with what has been deduced on the basis of direct observational study of deficiency mosaics (Bird and Fahmy, 1953), that deficiencies induced by the alkylating mutagens are mainly due to failure in gene reproduction rather than elimination of chromosome parts through breakage and reunion.

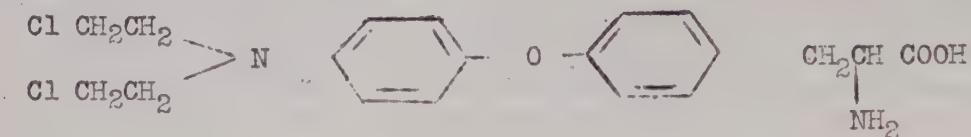
Fahmy, O. G., Fahmy,  
Myrtle J., and Purdom,  
C. E. The mutagenicity  
of the amino acid mustards.

property does not seem to be shared by the other biological alkylating agents, including the related phenyl-carboxylic mustards. The question then arose whether the above-mentioned selective mutagenicity is a special peculiarity of the phenylalanine mustard of whether it is a general feature of the mustard derivatives of amino acids.

It has been shown (see note above, and Fahmy and Fahmy, 1955c) that the "mustard" derivative of phenylalanine is most effective in the induction of visible (morphologically detectable) mutations. This

Attempts have been made, therefore, to test the mutagenic properties of the mustard derivatives of amino acids related to phenylalanine. A beginning

has been made by analyzing the genetic properties of *p*-N-di(chloroethyl) phenoxy-phenylalanine.



The sodium salt of the acid was dissolved at the required concentration in isotonic saline (0.4%) and was injected intra-abdominally into adult males. Concentrations of  $1.2-0.6 \times 10^{-2}$  M of the compound were used, and the volume injected varied from 0.2-0.4  $\mu$ l of solution per male. In each experiment 100-200 males were treated, and the same volume of solution was injected per male. The average dose per male in each experiment was calculated from the concentration of the injected solution and the volume received. The progeny of the treated males was fractionated by repeated mating to fresh virgin females every 3 days. The table below shows the variation in the sex-linked recessive lethal rate in the successive broods and with increase of dose.

Dose per male M x 10 <sup>-6</sup>	Brood								Total	
	I		II		III		IV			
	chr.	% <u>l</u>	chr.	% <u>l</u>	chr.	% <u>l</u>	chr.	% <u>l</u>	chr.	% <u>l</u>
1.5	487	0.6	470	2.1	97	6.2	25	4.0	1079	1.9
2.3	464	2.4	453	2.4	20	5.0	-	-	937	2.5
2.4	635	2.2	621	2.6	720	3.2	83	1.2	2059	2.6
2.7	467	2.1	487	3.7	55	5.5	11	0.0	1020	3.0
4.6	62	6.4	30	3.3	-	-	-	-	92	5.4

It can be seen that the phenoxy derivative is definitely mutagenic, though its activity is decisively lower than that of the phenylalanine mustard (see DIS-27 and -28). For example, an average dose of  $2.7 \times 10^{-6}$  M per male of the phenylalanine mustard gave a mutation rate of 9% sex-linked recessive lethals, as compared to 3% for the same molar dose of the phenoxy derivative.

Most interesting, however, is the fact that the phenoxy derivative seems to be practically ineffective in the induction of viable chromosome rearrangements. No viable translocations (between autosomes 2 and 3) have been detected among 1948 tested sperm treated with a range of concentrations from  $1.5$  to  $4.6 \times 10^{-6}$  M per male. In comparable tests for recoverable fragments, only one sperm out of the 3109 tested gave a mosaic hyperploid female.

These results are of interest in relation to the problem of the nature of recessive lethals. The fact that the phenoxy-phenylalanine mustard induces recessive lethals but practically no chromosome breaks (leading to viable rearrangements) argues against the theory that all recessive lethals are restituted chromosome breaks.

The efficiency of the phenoxy-phenylalanine mustard in the induction of visibles is still under investigation. The results so far, however, suggest that the incorporation of the phenoxy group in the molecule does not affect the activity on the morphogenesis loci appreciably. In a sample of 2059 X chromosomes treated by the phenoxy derivative, the ratio of visibles to lethals was found to be 0.26, which is of the same order of magnitude as that ascertained for the phenylalanine mustard (viz., 0.23).

Falk, R. A chromosome for the detection of the locus of lethals induced in the Muller-5 chromosome.

A common test for the rate of recessive X-chromosome lethals is the Muller-5 technique. It is sometimes of interest to modify the test and to induce the recessive lethals in the M-5 chromosome.

Localization of the mutations induced in the M-5 chromosome can be achieved by a special stock produced by crossing over between the M5ry chromosome ( $y\ sc^{S1}\ InS\ w^a\ sc^8$ ; Lüning, 1952, *Acta Zool.* 33: 193-207) and the M5w<sup>X</sup> chromosome ( $sc^{S1}\ B\ InS\ w^X\ sc^8$ ; Lüning, 1954, *DIS-28*: 132). Among the progeny of the M5ry/M5w<sup>X</sup> heterozygotes the desired genotype was  $y\ sc^{S1}\ InS\ w^X\ sc^8$ , called M5co (Muller-5 crossing over). From these flies the stock was obtained. From the crosses M5ry/M5w<sup>X</sup> and M5/M5co the standard distances have been determined as follows:

	M5ry/M5w <sup>X</sup>		M5/M5co		Total	
	No.	%	No.	%	No.	%
Interval y-B	178	15.1	340	18.4	518	17.1
Interval B-w	496	42.0	856	46.4	1352	44.7
Totals	1181		1844		3025	

The efficiency of M5co as a crossing-over inhibitor is shown by the progeny of the cross M5co/ec ct v f x M5co/Y:

♀		♂	
y w <sup>X</sup>	+	ec ct v f	y w <sup>X</sup>
232	321	243	232

Thus among 1028 flies no crossover was found. The viability of the M5co chromosome is satisfactory.

Farnsworth, M. W. Effect of homozygous Minutes on development of *D. melanogaster*.

Homozygotes of M(1)o, M(2)1, M(2)1<sup>2</sup>, M(3)w, M(3)B, and M(3)124 were studied to ascertain the underlying causes of lethality and to determine if these are in

any way related to one another. It was found that M(2)1 individuals die in the egg stage, whereas in the other stocks investigated death occurs in the first larval instar. Study of sectioned embryos has revealed that the various homozygotes are very similar to one another in that all present the same general pattern of anomalies. The most extreme of the series was M(2)1. In these embryos, the midgut does not lengthen normally and yolk utilization is greatly impaired, since masses of yolk remain in the gut even at 22 hours (normal hatching time). Other structures, such as the central nervous system and cephalopharyngeal apparatus, are frequently disorganized, although this is not always the case. A striking similarity has been observed between homozygotes of M(2)1 and M(4). With respect to the larval lethals--that is, M(1)o, M(2)1<sup>2</sup>, M(3)w, M(3)B, and M(3)124--in general, all are characterized by abnormalities of the midgut with accompanying slowness of yolk withdrawal. Such larvae are almost indistinguishable from one another. The Minutes so far investigated occupy loci on all four chromosomes, yet the homozygotes, as well as the heterozygotes, appear to be superficially alike, differing primarily in the severity and extent of the similar anomalies present.

Fox, A. S., Herskowitz, I. H., and Schalet, A. Negative mutagenic activity of rabbit antisera to the antigens of *Drosophila* adults.

1954). These tests were performed with microorganisms, however, which for various physiological reasons might not be responsive. Additional tests, utilizing the sperm-bath technique (Herskowitz, Genetics 40: 76, 1955), have consequently been performed with *D. melanogaster* to determine if antisera produce visible or recessive lethal mutations anywhere on the X chromosome.

Rabbit antisera were obtained by immunization with whole, homogenized flies (Fox, J. Immunol. 62: 13, 1949). These were tested for titer and specificity. All antisera were of high titer. Anti-Oregon-R sera possessed a variety of antibodies directed against antigens presumably produced under the control of numerous loci. Anti-v<sup>48a</sup> sera possessed, in addition, antibodies against three antigenic components specifically associated with the *vermilion* locus (Barish and Fox, Genetics, in press).

Of 90 Dasc females inseminated by Oregon-R males and treated with anti-Oregon-R serum, 40 gave offspring. A negative result for X-linked recessive lethals was obtained (0 lethals in 208 tests). After anti-v<sup>48a</sup> serum was administered to 115 sc<sup>8</sup>.Y/y f::; bw<sup>D</sup> females inseminated by Oregon-R males, 3883 male offspring were produced. None showed a bilateral modification of the wild-type phenotype which proved heritable; only two males were mosaic, one had some Minute bristles, the other had one bw<sup>+</sup> eye. These results for visibles are considered negative.

(Part of this investigation was done with the aid of a grant received for work of H. J. Muller and associates from the American Cancer Society, on recommendation of the Committee on Growth of the National Research Council. The work was also aided by grants to Allen S. Fox from the American Cancer Society and the National Cancer Institute of the National Institutes of Health.)

Freire-Maia, Newton  
Chromosome mutations in natural populations of *D. ananassae*.

Through the analysis of larvae from about 1400 females of *D. ananassae* collected in domestic habitats, from northern Brazil to Argentina, during the period 1951-1955, we have found 19 paracentric inversions (5 in IIR, 5 in III, 3 in IIIL and 6 in IIIR), 5 pericentric inversions (4 in chromosome III and 1 in chromosome II), one translocation (IIIR-IIR), one deletion of several bands (IIIL), two transpositions (II and III) and a great number of different "extra bands" in heterozygous as well as in homozygous condition at each chromosome tip. The only type of variation found in the X chromosome was these "extra bands." Also, it is interesting to point out that none of the inversions we have found in domestic species was found in the X chromosome. This is a quite different situation from that found in wild species.

*D. ananassae* presents qualitatively a chromosome polymorphism larger than all the other *Drosophila* species so far studied, having a number of inversions per larva (about 1.4) higher than the other domestic species. Although the number of pericentric inversions detected in its populations is the highest found in the genus, the total frequency of these inversions

Recent tests for the mutagenicity of antibodies have yielded uniformly negative results (Fox and Zieber, Genetics 37: 581, 1952; Fox, unpublished; Ryan, Fried, and Gonzales, Am. Nat. 87: 383, 1953; Markert and Owen, Genetics 39: 818, 1954).

in heterozygous condition is not high (0.6%) when compared with D. robusta (Carson and Stalker, 1947).

Despite all its store of chromosome variation, D. ananassae seems to be ecologically less versatile than other domestic species with lesser or absolutely no chromosome polymorphism. Moreover, it has been impossible until now to find any clear correlation between inversion-morphism and ecological versatility. Nevertheless, before any final conclusion can be drawn regarding this point, it is absolutely necessary to have precise information on the ecology of the species. Without knowledge of these data, it will always be possible to suppose that some species (like D. simulans and D. hydei), practically without chromosome polymorphism, are widespread and common not because they occupy several different ecological niches, but only because the few they exploit are also widespread and common. In the same way, one can interpret the fact that a highly polymorphic species like D. ananassae is not ecologically so versatile because it occupies many different but rare ecological niches (da Cunha, 1955).

The "extra bands" found are similar to those first described by Kikkawa (1938), but their number in South American populations is very large. It suffices to say that in many of the samples analyzed, the mean number of these cytologically terminal "duplications" per larva is higher than one. This character is not found in every larva, but it is not uncommon to find 2 or 3 "extra bands" in the same individual. Some of these "duplications" present a typical heterochromatin aspect, and sometimes they are seen in an end-to-end pairing.

Freire-Maia, Newton  
Inversions in wild and  
domestic populations of  
D. nebulosa.

The chromosome variation in thirty-four Brazilian populations of D. nebulosa has been analyzed through examination of the salivary-gland chromosomes of  $F_1$  larvae from crosses of collected samples with

flies from the Standard strain from Del Rio, Texas, described by Pavan (1946). Thirty-one gene arrangements have been found in chromosome III as a result of combination of eleven different inversions. Of these 31 arrangements, 8 were found only in the north, 11 only in the south, and 12 in both regions. Among the samples from twenty-six localities in the south, 4 were homozygous for inversion A, 2 for inversion C, and 14 for both. In none of the samples from the eight northern localities were these inversions present in homozygous condition. Furthermore, some inversions were found only in northern samples, although others were present only in southern ones.

A small sample of 19 females collected in an orchard in Boa Esperança, Minas Gerais, was also analyzed, through examination of one larva from each female, and the inversions A, B, C, F, G, and H were found. The mean number of heterozygous inversions per larva proved to be relatively small (0.89) and similar to the means found by da Cunha, Brncic, and Salzano (1953) in samples from a desert (caatinga) region. As a domestic habitat seems to be ecologically less heterogeneous than the natural non-desert ones, our data are in accordance with the hypothesis developed by Dobzhansky, Burla, and da Cunha (1950).

We have analyzed also, by the same method, a smaller sample from Grutas, Argentina, and found only the inversions B, G, and H, the mean number of heterozygous inversions per larva being 0.60. This datum agrees very closely with the 0.62 found by da Cunha, Brncic, and Salzano (1953) in another Argen-

tine locality "presumably near the margin of the distribution of the species," and also seems to confirm the same hypothesis.

In both these last two samples, the gene arrangement h (Standard) was present in frequencies much higher than the corresponding inverted segment (H). The frequencies of the three genotypes HH, Hh, and hh were in accordance with the Hardy-Weinberg formula.

Freire-Maia, Newton, and Freire-Maia, Ademar. Race differentiation in a domestic species--the case of D. kikkawai.

Offspring of light females inseminated in nature; The most important facts may be summarized as follows.

1) Northern populations generally show frequencies of the gene for light pigmentation significantly higher than those of southern populations, although in both regions the light form is the commonest. No cline has been observed, but only sharp differences between some localities. The frequencies of light females, from the more northern localities to the more southern ones, are: 84% (N=25), 81% (N=26), 89% (N=46), 80% (N=313), 85% (N=20), 92% (N=25), 88% (N=465), 59% (N=346), 79% (N=28), 62% (N=79), 63% (N=24), 57% (N=70), and 76% (N=66).

2) Some localities not very far apart present populations with significantly different gene frequencies. A striking situation is that of Morrétes and Paranaguá, only about 30 km apart, which have frequencies of light females of 88% and 59% respectively ( $\chi^2=8.80$ ). The frequencies of the allele for light pigmentation calculated from the data obtained with the males (method no. 3) are 94% and 71%, respectively. This difference is highly significant ( $\chi^2=114.28$ ).

This fact is very interesting, because it is proof that even relatively close populations of organisms capable of being easily carried by man (mainly through fruit transportation, an intense activity in the region just referred to) may undergo racial differentiation, probably through the action of natural selection.

3) The data obtained through the use of method 3 show that the frequencies of the three genotypes are always in accordance with the binomial square law. The following examples illustrate this point:

Localities	Homozygous dark (AA)	Heterozygous dark (Aa)	Light (aa)	Total	Chi-square
Morrétes	1	35	289	325	0.01
Paranaguá	18	95	110	223	0.16
Itajai	0	18	31	49	2.48

4) The results obtained through method 2 also show a genotype distribution according to the Hardy-Weinberg formula, as if each female were inseminated practically only once and as if no great differences existed in the degree of sexual activity of males of the three genotypes.

We have made an analysis of the problem of color morphism in some Brazilian populations of D. kikkawai Burla (previously referred to as D. montium de Meijere) in three different ways: (1) examination of collected females; (2) analysis of the

Frydenberg, Ove. Survey of  
Danish Drosophila species.

species which also according to modern taxonomy are referred to the genus *Drosophila*. During the years 1953-54, Frydenberg collected approximately 16,000 *Drosophila* specimens all over Denmark, using mostly ordinary fermenting-banana bait in modified Patterson cans. At the same time, the *Drosophila* collections kept in the Zoological Museum of Copenhagen were studied. The following species were caught (the number of specimens is given): (1) *D. deflexa* Duda, 104; (2) *D. busckii* Coq., 13; (3) *D. melanogaster* Meig., 1068; (4) *D. simulans* Sturtevant, 4; (3-4) *melanogaster* or *simulans* females, 16; (5) *D. obscura* Fall. - *D. obscuroides* Pomini, 2044; (6) *D. silvestris* Basden, 294; (7) *D. tristis* Fall., 16; (8) *D. ambigua* Pomini, 334; (9) *D. subobscura* Collin, 7370; (10) *D. transversa* Fall., 119; (11) *D. phalerata* Meig., 3228; (12) *D. limbata* v. Roser, 5; (13) *D. littoralis* Meig., 71; (14) *D. testacea* v. Roser, 16; (15) *D. funebris* Fabr., 550; (16) *D. hydei* Sturtevant, 20; (17) *D. immigrans* Sturtevant, 584; (18) *D. confusa* Staeger = *D. vibrissina* Duda = *D. grischuna* Burla, 21; (19) *D. fenestrarum* Fall., 44; (20) *D. forcipata* Collin, 1. All the above species were taken outdoors; *D. funebris* and *D. melanogaster* were frequently found indoors. *D. immigrans* and *D. subobscura* were each caught once indoors.

Only one of Zetterstedt's species, viz., *D. picta* Zetterstedt, was not found. Hence the Danish *Drosophila* fauna now comprise 21 species. The results are to be published shortly.

Fuscaldo, Kathryn. X-ray induction of lethals in the X chromosome of *D. melanogaster*.

A simple technique has been devised for detecting the effect of X-radiation in producing lethals in the X chromosome of *D. melanogaster*, by determining the sex ratio of the progeny of X-rayed females. Virgin females of Ore-R stock, one to eight days after eclosion, were subjected to 3000 r. In series I, a Kelecut unit was used, giving 100 r in 3 1/2 minutes at 15 milliamperes, 100 kilovolts at 36.5 cm; in series II, a Loreloo unit was used, giving 180 r per minute at 8 milliamperes and 140 kilovolts at 3.8 cm (an .85 aluminum filter was used). The irradiated females were mated to Ore-R males in pair matings and the sex ratio of the offspring was determined from those which developed from eggs laid within the first 8 days after treatment. The controls were untreated Ore-R females mated to Ore-R males. All material was kept at 25° C. The control series gave 537 male, 549 female offspring, showing a close approximation to a 1:1 ratio, with  $\chi^2 = .132$  and  $P = 70\%-80\%$ . Series I gave 683 male, 804 female offspring, differing from a 1:1 ratio by 8.14%, with  $\chi^2 = 9.84$  and  $P$  less than 1%. Series II gave 417 male and 510 female offspring, differing from a 1:1 ratio by 10.03%, with  $\chi^2 = 9.32$  and  $P$  less than 1%. (In these experiments, no discrimination was made between the kinds of lethals produced, but there were probably few translocations, as Glass has shown that translocations are rare when oocytes are irradiated.) This method indicates that X-ray induction of X-chromosome lethals can be measured by the effect upon the sex ratio of the next generation (a reduction of one generation from the technique of X-raying the male), and that this measurement is repeatable.

Glassman, Edward, and  
Glass, Bentley. Kynurenine  
formamidase in *Drosophila*.

The enzymes involved in the metabolism of tryptophan and some of its derivatives in *Drosophila* have been investigated. Neither

tryptophan nor kynurenine was found to be metabolized enzymatically in cell-free extracts of various stages (although kynurenine condenses chemically with tyrosinase-produced quinones in crude larval extracts, see following note). On the other hand, the enzymatic hydrolysis of N<sup>1</sup>-formylkynurenine to kynurenine and formic acid occurred readily. This enzyme, kynurenine formamidase (formylase), was unusual in that it did not hydrolyze formylanthranilic acid; the enzyme extracted from rat liver or *Neurospora* does attack this compound.

Kynurenine formamidase was assayed in 20 stocks of *D. melanogaster* and 6 stocks of *D. virilis*. In all cases, including those stocks which cannot make kynurenine (viz., the vermilion pseudoalleles of *D. melanogaster* and cd of *D. virilis*) or else make this compound in subnormal amounts (claret and ruby), the levels of enzyme activity (based on mg of protein) were comparable to that in the wild type. Levels of enzyme activity were lower in *D. virilis* than in *D. melanogaster*, however. It is concluded that those mutants which affect kynurenine production probably do so through the tryptophan-peroxidase system.

Glassman, Edward, and Glass, Bentley. The relation between tyrosinase-produced quinones and the disappearance of kynurenine in larval extracts of *Drosophila*.

these compounds was taking place, but detailed analysis was not pursued.

A reinvestigation of this phenomenon, undertaken with the expectation that perhaps hydroxylation of kynurenine was occurring, revealed that this was not the case. Instead, the requirement for oxygen is due to the oxidation of tyrosinase substrates which are present in crude larval extracts, and which are converted to quinones. These quinones react nonenzymatically with kynurenine or 3-hydroxykynurenine through their aromatic amino groups and give rise to a pigmented complex.

The evidence for this is mainly as follows: (2) other compounds which contain an aromatic amino group, such as anthranilic acid, p-aminobenzoic acid, and 3-hydroxyanthranilic acid, will also respond like kynurenine in crude larval extracts, whereas closely related compounds, such as p-hydroxybenzoic acid and 2,3-dihydroxybenzoylalanine, which are not aromatic amines, do not react; (b) none of these compounds increases the oxygen uptake of crude larval extracts; and (c) removal of endogenous substrates will eliminate the disappearance of these aromatic amines and the production of pigment. When either tyrosine, phenol, 3,4-dihydroxyphenylalanine (DOPA), or catechol are added back, the extracts will again react with amines and form pigment. These results have been repeated using extracts of blowfly larvae (*Phormia regina*) and mushroom tyrosinase.

The identity of the endogenous quinone is not certain, but only DOPA-quinone is as specific as it is for aromatic amines. Other quinones derived from catechol or tyrosinase substrates found in insects (Hackman *et al.*, 1948, *Biochem. J.* 43: 474) react with either aliphatic or aromatic amines.

The products obtained using aromatic amines are fundamentally similar to previously described "aminoquinone" pigments, which are derived from quinones and various aliphatic amines or amino acids (see Hackman and Todd,

Tatum and Beadle (1938, *J. Gen. Physiol.* 22: 230) reported that unheated larval juices could inactivate the v<sup>+</sup> or cn<sup>+</sup> substances (kynurenine and 3-hydroxykynurenine, respectively), but only in the presence of oxygen. These workers concluded that an enzymatic oxidation of

1953, Biochem. J. 51: 631, for references). Any differences which exist can be reconciled on the basis of structure. There may be some relation between "aminoquinones" and the brown eye pigment of *Drosophila*, but this is purely speculative. At any rate, because of the presence of these reactive quinones in crude larval extracts, biological and biochemical investigations involving larvae would do well to take these compounds into consideration. The mechanism which keeps these quinones "under control" during melanogenesis in living systems is not known, but is certainly of fundamental importance.

Goodwin, S. W. Effect of ring-gland transplantation on nonpupating tumor-bearing larvae.

Experiments were performed in an attempt to determine whether failure to pupate is causal to lethality in tumorous larvae or if these two phenomena are concomitant and have a common cause. Ring glands from nontumorous larvae were transplanted into tumorous larvae to see if the host larvae would pupate and, if so, whether or not pupation would be followed by death. Donors were late-third-instar larvae of a Canton-S stock, which also supplied the control hosts. The following results were obtained:

<u>Hosts</u>	<u>Number</u>	<u>Number of Pupations</u>
tumorous larvae	26	0
control larvae	13	

It is concluded from these results that failure to pupate is not due to a disorder of the hormones of the ring glands of tumorous larvae.

Green, M. M. On the lethal interaction of the mutants purple and eyeless in *D. melanogaster*.

Confirmed, since the following genotypes proved to be quite viable and fertile in both sexes: *ci ey<sup>R</sup>*; *pr, ey<sup>2</sup>*; *pr* and *ey<sup>D/+</sup>*; *pr*.

Hadorn, E., and Kürsteiner, R. Biochemical pleiotropy in excretion products.

There are more different substances present in the mutant, and some of the compounds common to both genotypes are much more concentrated in *w* than in *+*. On the other hand, no significant differences were found between the excretion material produced by *sepia* (*se*) and the normal wild type. A report will appear in the next volume of the Arch. Jul. Klaus-Stiftg.

Hannah, A., and Strømnes, Ø. Extra sex comb mutants in *D. melanogaster*.

Two mutants have been described in *D. melanogaster* which induce the formation of sex combs on the second and third legs of the male flies. The first, *esc* (extra sex comb), described by Slifer (1942), is a second-chromosome recessive, homozy-

In the *l(l)7* strain of *D. melanogaster* a genetically controlled tumor is associated with failure to pupate and death after a prolonged third-instar stage. (Lewis, J. Exp. Zool. 126: 255-276). Transplantation

gous-sterile mutant. In addition it has relatively low penetrance and variable expressivity, both factors being affected by various environmental conditions. The second, *Pc* (Polycomb), described by P. Lewis (DIS-21), is a third-chromosome dominant, homozygous lethal and with phenotypes and responses to environmental conditions comparable to those of *esc*.

Recently two more "extra sex comb" mutants have been found, a second-chromosome recessive by Strömnaes and a third-chromosome dominant by Hannah. In addition, G. Lefevre, Jr. (personal communication) reports that males with sex combs on the second and third legs are not uncommon phenocopies in flies of certain stocks irradiated as larvae; most of the flies tested so far have been sterile.

*Scx* (Extra sex comb, Hannah 53b, 3-48 $\ddagger$ ) arose spontaneously as three males among 1354 male offspring in a cross of *In(1)dl-49*, *y*, *Hv*, *v<sup>o</sup>*, *m<sup>2</sup>*, *f*/*xc<sup>2</sup>* sn females (aged before mating) and *X<sup>c2</sup>* males. Although these strains have been used extensively for several years, this was the only time that males with extra sex combs were observed. Like *Pc*, *Scx* is dominant, homozygous lethal; and as in both *esc* and *Pc* the penetrance and expressivity are variable and influenced greatly by environmental conditions. The *Pc/Scx* compound is not lethal, penetrance is considerably higher; the average number of teeth in the comb of the male second leg is 11.2, and in the third leg it is 8.3. However, in *esc/esc*; *Scx/+* compounds the penetrance is also increased and the average number of teeth per comb is 11.2 in the second leg, 7.6 in the third.

P. Lewis had previously reported that *Pc* is between *th* and *p*. By means of crossover tests, with *ru*, *h*, *st*, *pP*, *ss*, *e<sup>s</sup>*, both *Scx* and *Pc* were found to be located between *st* (44.0) and *pP* (43.0), probably just to the left of *pP*. The lethal associated with *Scx* is also just to the left of *pP*. These results suggest that *Pc* and *Scx* may be allelic. This is substantiated by the fact that there appears to be no crossing over between the two mutants. However, other evidence indicates that this may not be the case, for they behave differently in compounds with the *Antennapedia* mutants. *Scx/Antp<sup>Yu</sup>* and *Scx/Antp<sup>B</sup>* are lethal, whereas *Pc/Antp<sup>Yu</sup>* and *Pc/Antp<sup>B</sup>* are viable and fertile.

Preliminary cytological studies of *Pc* and *Scx* indicate there is no chromosomal change associated with either (Hannah; Mickey, personal communication). *Antp<sup>Yu</sup>* and *Antp<sup>B</sup>* have not been localized genetically, but the former is a complex translocation with one of the breaks at 83E-F and the latter is an inversion with one break at 84A in the salivary chromosome map (E. B. Lewis, personal communication). Mutants with similar phenotypes but usually called *aristapedia* dominants, have been reported in the literature; if they are accompanied by a chromosomal change, one break of the inversion or translocation is in the 83-84 region. The cytological locus for *p* has tentatively been put at 85A-C (C. Ward, personal communication). From these results it seems likely that the cytological locus of *Scx* may be in the same region as *Antp*, that is, in sections 83-84. Until there is more cytogenetic evidence that *Pc* and *Scx* are alleles they can be considered either as independent loci or as members of a pseudoallellic locus.

The second-chromosome mutant, *esc*, is associated with *In(2L)t* (E. B. Lewis, personal communication). Evidence from crosses of *esc* and *Cy* (a similar inversion) suggests that the locus for *esc* is not at the left break point; assuming that the locus for *Cy* is 3.5 $\ddagger$ , *esc* is somewhere to the right and perhaps within the inversion.

The allele to esc, esc<sup>2</sup> (extra sex comb, Strømmeas 53f, left arm 2) arose spontaneously as four males in a selection experiment where the foundation stocks were made up from crosses between eight wild-type laboratory stocks. Whereas esc males are sterile, the esc<sup>2</sup> males are fertile. esc/B1 ♀ x esc<sup>2</sup> ♂ gave in the F<sub>1</sub> males with extra sex combs. No translocation has been found in the esc<sup>2</sup> stock, but there are indications of the presence of a lethal floating in this stock. Crossovers have been obtained between esc<sup>2</sup> and al, dp, b, pr, c, px, sp chromosomes. Further localization is in progress.

In all the extra sex comb mutants studied morphologically the effect is to change the second and third legs into first legs. In the male the first tarsus of the second and third legs becomes shorter and the chaetotaxal pattern of the first tarsus and the distal end of the tibia are changed into a male first leg pattern, the extent of change being dependent upon the number of extra sex comb factors present. The final phenotype is influenced greatly by various environmental factors. The changes in the female second and third legs are comparable to and parallel with the changes in the male legs, that is, they are changed into female first legs.

Henke, H., Hähne, G., and  
Hünkel, H. A. Tests of two  
 new mutagenic compounds with  
*D. melanogaster*.

Among the chemical substances that occupy an important position in the treatment of malignant processes, the nitrogen mustards show a remarkably high mutagenic effect. Continuing our investigations on the induction of mutations by cytostatic agents, we have determined the mutagenicity of a new nitrogen mustard, methyl-bis-( $\beta$ -chloroethyl)-amine-nitrogenoxide-hydrochloride (N-oxide-mustard), and of the compound 2:5-bis-ethylene-imino-benzochinone-1:4 (Bayer G 4073), another cytostatic substance recently produced.

About 0.5 mm<sup>3</sup> of the aqueous solutions of these compounds was given to the males by intraabdominal injection one day before copulation. Additionally we investigated the effect of Bayer G 4073 orally taken up from a specially prepared yeast medium by larvae and hatched males before mating. The rates of recessive sex-linked lethals were determined by the Muller-5 technique and are shown in the table. (See p. 124.)

It is obvious that N-oxide-mustard causes a rate of lethals 8 to 14 times as high as the rate of spontaneous mutations. This effect was no higher than that of N-mustard. The cytostatic compound Bayer G 4073 increases the percentage of lethals to a value that is only 3 or 4 times the control rate. When fed to the flies this agent has no mutagenic effect at all. It may be that this method of application does not provide a high enough concentration for induction of mutations.

The compounds differed in toxicity. N-oxide-mustard showed relatively slight harmful effects. After injection of a 1% solution, 29% of the flies died within 24 hours. Bayer G 4073 (0.5%), however, caused death of 64% of the animals within 12 hours after injection.

(Table on following page.)

Compound	Concentration (%)	Kind of application	No. of chromosomes	No. of lethals	% lethals
NaCl	0.75	abdominal injection	1187	4	0.34
N-oxide-mustard	2.5	"	571	16	2.80
	1.0	"	564	28	4.96
Bayer	0.5	"	724	10	1.38
G 4073	0.1	"	503	6	1.19
	0.5	feeding	1121	4	0.36

Herskowitz, I. H. The mutagenicity of "triazine" administered in sperm baths.

When 2:4:6-tri(ethyloneimino)-1:3:5-triazine ("triazine") was injected into adult males, as much as 18% (48 lethals in 266 tests) sex-linked recessive lethal

mutations were obtained (Bird, M. J., J. Genet. 50: 480-485, 1952). However, when a triazine solution (about 1%) was given as a postcopulatory vaginal douche only 6.8% of the 2055 chromosomes tested had such mutations (Herskowitz, Rec. Genet. Soc. Amer. 24; and Genetics 40: 574, 1955). Since it was shown that sperm baths are a more effective way of treating mature sperm than postcopulatory vaginal douches, at least in the case of formaldehyde (Herskowitz, Genetics, 40: 76-89, 1955), the same solution of triazine was given in sperm baths to 46 females of which 12 gave offspring. There were 11 lethals found in 63 tests (17.5%), a mutation frequency which is significantly higher than the rate obtained after the douche treatments and of a magnitude comparable to the highest mutation rate so far reported for this substance. (This work has been supported by a grant received for work of H. J. Muller and associates from the American Cancer Society, on recommendation of the Committee on Growth of the National Research Council.)

Herskowitz, I. H., and Abrahamson, S. Differences in mutability of stocks of *Drosophila* with formaldehyde feeding.

When different strains or stocks of the same species are treated with a mutagenic agent the frequencies of recessive lethals induced are sometimes very different. In the present experiments with *D. melanogaster*, all run simultaneously, a 10% aqueous solution of formaldehyde was pipetted onto the surface of food medium contained in half-pint bottles, in an amount which would have made the concentration in the medium 0.2% if diffused throughout. In this medium 24-48-hour-old individuals of either the wild-type Oregon-R stock, the *tu<sup>48</sup>* stock, or the *Hx* stock were developing. The last two stocks had originated independently as mutants in the *Basc* stock, the *tu<sup>48J</sup>* stock having since lost *B*, whereas the *Hx* stock had since had at least the *X* chromosome replaced by that of the Oregon-R. Random samples of males surviving this treatment were obtained, each *tu<sup>48J</sup>* male being mated to two untreated Oregon-R females and each of the other males to two untreated *Basc* females. Up to 12 sperm from each male were tested for sex-linked recessive lethal mutations.

The mutation rates obtained in the different stocks were for Oregon-R 3.5% (26 lethals in 736 sperm tested), for *Hx* 3.1% (37 lethals in 1074 tests), and for *tu<sup>48J</sup>* 10.7% (44 lethals in 410 tests). The *tu<sup>48J</sup>* stock, therefore, gave a lethal frequency three times that of the other two stocks

tested. This result is consistent with the fact that the  $tu^{48j}$ -bearing male has a considerably lower viability than males of the other two stocks, so that many induced mutants that were only "detrimentals" in the latter would be lethal in the former, and with the work of Meyer and of Iyengar, unpublished, who previously obtained evidence that differences in the frequency of induced lethals can be on the phenotypic level, caused by viability differences between individuals of the classes compared. In view of the fact (Abrahamson and Telfer, Rec. Genet. Soc. Amer. 23: 28-29, and Genetics 39: 955-956, 1954) that sperm delivered the first day after eclosion have a greater number of X-ray-induced mutations in them than do sperm delivered the next day (and probably in the succeeding copulation), the results also might have been obtained because  $tu^{48j}$  males mated fewer times, on the average, than males of the other two stocks (partly because the  $tu^{48j}$  male is less aggressive than the other males, and partly because the Oregon-R female is relatively more evasive than the Basc females to which the Hx and Oregon-R males were mated).

These results emphasize the importance of the intra-variety genetic composition for chemical mutagenesis in *Drosophila*.

(This work has been supported by a grant received for work of H. J. Muller and associates from the American Cancer Society, on recommendation of the Committee on Growth of the National Research Council.)

Herskowitz, I. H., and Abrahamson, S. The effect of X-ray intensity on the rate of sex-linked recessive lethal mutations induced by treatment of *Drosophila* oocytes.

after which they were treated. A total of 3264 r was delivered to the females, either in an intense treatment (continuously in 2 min. 36 sec. at the rate of 1260 r/min.) or in a protracted one (interruptedly in 4 hrs. 56 min. in 16 irradiations each 6 min. long at the rate of 34 r/min., with the first seven and last seven nonirradiation intervals each of 10 min. and the eighth interval of one hour). After irradiation each female was placed in a separate vial with two Basc males, and transferred to a new culture vial after 2 days, and the parents were discarded at the end of the 4th day after completion of the irradiation. The intense treatment gave  $4.4 \pm 0.57\%$  lethals (57 lethals in 1303 tests) whereas the dilute gave  $2.0 \pm 0.25\%$  (66 lethals in 3275 tests). These values differ significantly ( $P < .003$ ).

Although other interpretations are not yet excluded, the data strongly suggest that as many as one-half of the lethals produced by the intense treatment are connected with multiple X-ray hits.

(This work has been supported by a grant received for work of H. J. Muller and associates from the American Cancer Society, on recommendation of the Committee on Growth of the National Research Council.)

Hinton, C. W., and Welshons, W. J. A spontaneous deficiency occurring in  $X^{c2}$ .

From the cross of  $y\ w\ spl\ sn^3/dl-49$ ,  $y\ v\ car$  females by  $X^{c2}$ ,  $cv\ v\ f/sc^8.Y$  males a single  $sn$  female appeared among 8524  $F_1$  females. On inbreeding by  $y\ w\ spl\ sn^3$

sc<sup>8</sup>.Y sibs, this sn female produced y w spl sn<sup>3</sup> daughters, w spl sn<sup>3</sup> sons, and sn daughters. A ring chromosome was observed in neuroblast metaphases. Examination of the salivary chromosomes revealed a deficiency with maximum limits extending from bands 7B3 to 7D22. This chromosome may represent a breakage product of a bridge formed at anaphase II in the testis of the parental X<sup>c2</sup> male.

Genetic tests have shown that the loci of ct, oc, and ptg are not included in this deficiency. Combining these data with those of Demerec et al. (1942, Carnegie Inst. Wash. Yr. Bk. 41: 191), the following more precise localizations can be made: sn between 7C4-5 and 7D22; oc and ptg between 7D22 and 8C1-2.

Jacobs, M. E. Phototaxis as isolating light and dark wild strains of D. melanogaster.

The bottoms of twelve 4" x 10.5" mailing tubes were cut out and a flue stop type gate was placed in the bottom of ten of them. The gates were made of thick round

plastic cover dish lids from a dime store. A disk cut from photographic film to fit the mailing tube was glued to each gate, and a stiff wire with an angular bend was fastened to each gate by thread passing through holes drilled in the gate. Cellophane windows were fastened to one end of two tubes (toward the light), and the remaining tubes were juttet behind these two with gates toward the light. Each tube was sprayed inside with water. Collars, made of store tape, were slid over the junctures. Collars and tubes were painted black. The lids were screwed on the last two tubes. Holes were cut in two tubes (fourth from the light) for admission of flies. The two rows were placed in a long covered cardboard box with two holes cut at one end for snug fitting of the cellophane end of the rows, where a fluorescent light was placed. To remove flies, the gates were closed and the lids were screwed on the tubes. (Jarring caused the flies to go toward the light plastic gate.) By bumping each tube into a large glass funnel through a cork, flies were removed to a bottle and counted.

Four light and four dark strains of melanogaster collected at Beaufort (North Carolina) were tested for phototaxis. When a half-pint bottle of culture medium was placed in each tube and equal numbers of two-hour-old light and dark males and females were placed in the tubes and left four days with the cellophane end of one row darkened, the first two tubes admitting light contained a preponderance of light flies and eggs, while for the rest of the tubes the converse held. A greater number of these females produced offspring of their kind than did those in the darkened control row, indicating endogamic mating due to the light gradient.

Judd, B. H. Interaction of w and p<sup>P</sup>.

Crosses of w with p<sup>P</sup> to give w<sup>+</sup>; p<sup>P</sup>/<sup>+</sup> heterozygotes show a non-wild-type eye color. The phenotype is only slightly

different from +, being a little more brown in color. This phenomenon does not appear to be due to a "near +" white allele in the p<sup>P</sup> stock, since the cross p<sup>P</sup> female with w male gives + males and brownish females. Several white alleles have been tested (w, w<sup>h</sup>, w<sup>bf</sup>) and all show this interaction.

Judd, B. H. Variegation in N<sup>264-12</sup>.

N<sup>264-12</sup> is described as showing variegation for w, rst, fa, dm, and with the Notch characters not always distinct.

Salivary-gland-chromosome analysis by Sutton shows the break in X to be between 3C5-6 and 3C7; therefore, this is an unusual case if variegation due to V-type position effect is occurring for fa, dm, and possibly Notch.

Analysis of this translocation shows that N/w spl is white-variegated and split. The white variegation is suppressed by an added Y chromosome, whereas split is not (Schultz reports nonsuppression of split with two additional Y chromosomes); the Notch character appears as frequently in XXY as in XX females. No variegation is seen for diminutive, though the thoracic hairs frequently show typical Notch characteristics.

Tests show that the Notch character is associated with the proximal part of the translocated X chromosome. For example N264-12L.w<sup>258-18</sup>R is never Notch, but does show variegation for w and rst; whereas w<sup>258-18</sup>L.N264-12R is always N, though it is deficient for only 3C5-6. w<sup>258-21</sup>L.N264-12R is often N at temperatures where w<sup>258-21</sup> rarely is. w<sup>258-21</sup>L.N264-12R is homozygous viable, and N males sometimes appear.

V-type position effect does seem to account for the w and rst variegation but not for spl or N in this translocation.

Kanehisa, T. A relation between incidence of "melanotic tumors" and the eye-color genes in D. melanogaster.

in concentrations of  $10^{-2}$  and  $10^{-3}$  M. An increase in tumor incidence was found in these cultures. The lowest effect of tryptophan was found in strains tu<sup>g</sup> and tu, and the effect increased in the following sequence: tu<sup>g</sup> = tu, v tu, cn tu, tu st. When v tu flies were cultured on medium containing kynurenin, the eye color of the genetically v flies became phenotypically wild-type red, and the tumor incidence of these flies fell and reached the low incidence found in the tu strain. These results show that the tryptophan metabolic changes in the course of the formation of eye-color pigments are intimately related with the incidence of melanotic tumor.

Kanehisa, T. An effect of indoleacetic acid on tumors in D. virilis.

that these tumors differed in appearance from the "melanotic tumors" of D. melanogaster. In my present experiment, flies were cultured on ground wheat and sugar media which contained indoleacetic acid in concentrations of  $10^{-2}$  M,  $10^{-3}$  M,  $10^{-4}$  M, or  $10^{-5}$  M. The effect of indoleacetic acid was obvious in the  $10^{-4}$  M concentration and obscure in each of the other concentrations. Abnormalities appearing as pale-blue, blackish aggregates were found in the body cavities of the adult flies 2 or 3 days after emergence. These abnormalities resembled in appearance those found in tumor flies which are homozygous for v bw, cn bw, or bw st genes, rather than the so-called "melanotic tumor" of D. melanogaster.

Kato, M. Induction of mutations by ultrasonic vibration in D. melanogaster.

The following strains--tu, v tu, cn tu, and tu st--were newly established from strain bw tu. Flies of these new strains and of strain tu<sup>g</sup> were cultured on

"synthetic" media containing tryptophan

and tu<sup>g</sup> were newly established from strain bw tu. Flies of these new strains and of strain tu<sup>g</sup> were cultured on

E. W. Hartung personally communicated to me that "melanotic tumors" were induced by indoleacetic acid in the bodies of D. virilis flies (Maruyama strain), and

that these tumors differed in appearance from the "melanotic tumors" of D. melanogaster.

Since 1952, experiments have been performed with doses of 450, 561, and 720 kc. Both dominant and recessive lethals have

been discovered, as well as a visible mutant with eyes of a brighter red than vermillion. The average rate of induced recessive lethals is 24.5%. No linear relationship has been found between dosage and frequencies of mutation.

Khishin, Aziz F. Effect of larval and pupal irradiation on survival and fertility.

It is well known that pre-imaginal stages of *Drosophila* are much more sensitive to direct damage by X-rays than are imagines.

For mutation experiments on these stages it is necessary to find a dose which, while giving an appreciable mutagenic effect, allows one to raise a sufficient number of  $P_1$  and  $F_1$  flies. As a preliminary to a study of the varying genetical response of male *Drosophila* germ cells to X-radiation (Khishin, 1955), survival and fertility of X-rayed larvae and pupae were determined at three dose levels. The data are presented in the table.

First-instar larvae were practically all killed by 2000 r; this is in agreement with a finding by Enzmann and Haskins (1938). With increasing larval age, survival rate increased; at all pupal ages it was normal even after irradiation with 2000 r. Fertility of the males during the first three days of imaginal life showed the opposite trend. It decreased from the first through the second and third larval instars, and males which had been irradiated as pupae with 1500 or 2000 r units were completely sterile. Even after pupal irradiation with 1000 r, fertility during the first three days was very low. In a separate experiment in which males were treated as pupae with 1300 r, hatchability was nil among 1100 eggs. In some series, late larvae which were ready for prepupation were sterilized by 1300 r. In all cases of larval and pupal irradiation, fertility was restored in later broods, usually after three days.

It does not seem possible to explain this temporary sterility after larval and pupal irradiation wholly by one of the two known causes of X-ray sterility: destruction of germ cells, and dominant lethality. The first could be ruled out by the finding that even during the completely sterile period the males transferred motile sperm to their mates. The second, dominant lethality, cannot account for the low fertility after larval irradiation. Dominant lethals are not usually produced in the premeiotic cells which constitute the larval testis. Moreover, the genetical effects on the larvae, as shown by the low frequencies of recessive lethals, were inconsiderable. In pupae, irradiation may produce dominant lethals and does in fact do so, as shown in other experiments. However, since 900 r units produced 7-8% sex-linked lethals in the most advanced germ cells of young pupae, 1500 r would be expected to produce not more than 12-13%. Unless the ratio of dominant to recessive lethals is very different when germ cells are irradiated in the pupa instead of in the imago, a dose of this mutagenic magnitude would not produce 100% dominant lethals.

It seems, therefore, that in the physiological environment of the old larvae and young pupae irradiation may damage germ cells in such a way that the spermatozoa which develop from the damaged cells are functionally ineffective. Such morphologically normal and motile, but nonfunctioning spermatozoa are also produced when larvae are reared on ethylene glycol (Bhattacharya, 1949). The restriction of the sterile period after irradiation of larvae and pupae to the first few days of reproductive activity shows that not all germ cells suffer this kind of damage. The stages which are sensitive to it seem to be mainly primary and secondary spermatocytes and early spermatids.

Stage	Age in hours	No. irra- diated	Emergence (%)			Fertility of the Males							
			1000	1500	2000	1000 r			1500 r			2000 r	
			r	r	r	No. tested	Fertile No.	%	No. tested	Fertile No.	%	No. tested	
larvae	40	190	57	41	1				14	10	74.5	1	1 100
	64	100	92	72	78	not tested*			10	4	40	11	3 27.3
	112	100	96	94	92				20	7	35	20	2 10
pupae	126	100	96	87	96	20	16	80	100	0	0	46	0 0
	150	100	92	94	100	20	2	10	40	0	0	60	0 0
	174	100	100	100	100	20	14	70	40	0	0	30	2 6.7

\* Fertility was normal.

Kikkawa, H., Ogita, Z., and Fujito, S. Problems concerning metal absorptivities in *Drosophila*.

The finding that each eye- or body-color mutant of *D. melanogaster* has a selective metal absorptivity has given us a clue to the solution of various problems concerning the nature and function of the gene.

Some preliminary results thus far obtained will be reported here.

a) Identification of homologous genes in different species: When homologous chromosomes or genes are identified in two different species which are unable to cross with each other, it is a commonplace method to compare analogous mutants with each other. Thus, Chino (1937) and Sturtevant and Novitski (1941) independently have published comparisons between *melanogaster* and *virilis*. The results of metal analyses obtained by us have revealed that some of their identifications may be adequate, but that others are not.

<i>D. melanogaster</i>	<i>D. virilis</i>	Result
yellow (Ti)	yellow (Ti)	Yes
white (Ni)	white (Ni)	Yes
vermillion (Fe)	vermillion (Fe)	Yes
garnet (Co)	apricot (Fe)	No
cinnabar (Co)	scarlet (Co)	Yes
brown (Co)	eosinoid (Cu)	No
scarlet (Cu)	cinnabar (Co)	No
ebony (Cu, Co, Fe)	ebony (Cu, Co, Fe)	Yes

b) Metal patterns in a heterozygous type: As shown in a previous paper (Kikkawa, Jap. J. Genet. 30: 46), a recessive gene responsible for metal absorptivity acts even in a heterozygous state. This fact has been demonstrated quantitatively, also. The following is an example.

Metal	Mutant types		
	+/+	+/w <sup>e</sup>	w <sup>e</sup> /w <sup>e</sup>
Cu	37	74	105
Ni	82	57	13

(p.p.m. of dry adult weight)

It is of interest that the amount of a given type of metal contained in a heterozygous type is nearly intermediate between those of two homozygous types.

c) Metal patterns in a double mutant: A double mutant consisting of two different types for metal absorptivity shows metal patterns of the two original types. This fact has been demonstrated by a quantitative method, as well as by a paper-chromatographic method. The following represents an example.

Metal	Mutant types		
	cn	cn, w	w
Co	108	44	12
Ni	15	66	119

(p.p.m. of dry adult weight)

It is of interest that the amount of a given type of metal contained in a double mutant is nearly intermediate between those of the two original types.

d) Metal patterns of pseudoalleles: Pseudoalleles in the white series of D. melanogaster are the most suitable examples for the purpose of this investigation. As far as our experiments show, they are divided into three or four principal types: (1) Ni type--w; (2) Cu-Co type--w<sup>e</sup>, w<sup>e2</sup>, w<sup>t</sup>, w<sup>bf2</sup>; (3) Fe type--w<sup>ap</sup>, w<sup>h</sup>; (4) Cu-Fe type--w<sup>ch</sup>, w<sup>ch</sup>-like (discovered by Kikkawa in 55g). It is to be noted that in the Cu-Co type and the Cu-Fe type, the eye color of females is darker than that of males, whereas in the Fe type the relation is reversed.

e) Metal patterns in phenotypic variants induced by chemicals: When kynurenine or 3-hydroxykynurenine is fed to v or v bw mutant larvae, the eye color of the adult fly becomes phenotypically normal or bw type. A similar phenomenon is observed when 3-hydroxykynurenine is fed to cn or cn bw mutant larvae. The metal pattern of such phenotypic variants varies according to the change in eye color, as shown in the following table. The detailed descriptions of these phenomena will be presented elsewhere.

Phenotype	Metal pattern
v (control)	Fe
Normal type of v induced by kynurenine or 3-OHkynurenine	Fe, Cu, Co, Ni
Normal (control)	Fe, Cu, Co, Ni
v bw (control)	Fe, Co
bw type of v bw induced by kynurenine or 3-OHkynurenine	Fe, Co
bw (control)	Co
cn (control)	Co
Normal type of cn induced by 3-OHkynurenine	Fe, Cu, Co, Ni
cn bw (control)	Co
bw type of cn bw induced by 3-OHkynurenine	Co

f) Biological effects of different metals on viabilities of *Drosophila* mutants: As described in DIS-28, page 125, the mutant larva absorbing a particular metal is liable to die on medium containing that metal in abundance. By utilizing this phenomenon, it is possible to examine biological effects of various metals on viabilities of *Drosophila* mutants. The following shows some data relating to this problem. Thirty larvae per strain were used for each experiment; the figures show numbers of survivors.

Donor metal	Concentration (mM)	y(Ti)	cn(Co)	Recipient w(Ni)	w <sup>ap</sup> (Fe)	e(Cu)
Pb	2.5	1	20	20	18	23
Zn	2.5	12	11	3	12	14
Mo	2.5	28	25	23	25	3
Mn	3.0	15	14	11	1	12
V	2.0	21	20	20	2	16

From this table, it is easily conjecturable that Pb is substituted for Ti, Zn for Ni, Mo for Cu, Mn and V for Fe. Effects of other metals can be examined by similar biological tests. The details will be described elsewhere.

g) Absorptivity of a certain chelating substance: It is very reasonable to assume that a mutation causing absorption of a particular metal from the medium may also bring about absorption of a chelating substance which is easily coordinated with that metal. Since xanthopterin or folic acid seems to be always associated with titanium, the synthetic material was added to the medium, and larvae of several mutant strains having different metal absorptivities were bred on that medium. After pupation, they were collected and examined by a paper-chromatographic method, as to whether xanthopterin or folic acid was absorbed by a certain mutant or not. The examination was of course checked by a control experiment. As shown below, mutant strains which are apt to absorb titanium contained xanthopterin or folic acid in their bodies abundantly. This fact seems to indicate that the absorptivity of a certain chelating substance is also dependent on the genetical constitution concerning metal absorptivity.

Chelating substance	Wild	y(Ti)	se(Ti)	Strain t <sup>3</sup> (Ti)	e(Cu)	w(Ni)	bw(Co)
Xanthopterin	+	++	*	*	-	*	*
Folic acid	+	++	++	++	-	-	-

\* Undetermined.

Kitagawa, O., and Moriwaki, D.  
Functioning of implanted ovaries  
in "Female-producing female  
(Sex-ratio-J)" of *D. bifasciata*.

(1953) suggested for "Sex-ratio." With a view to making this clear, the method of transplantation was applied. Larval ovaries of one genetic constitution, or (occhio rosso--autosomal recessive eye-color mutant), were implanted in SR-J larvae. Out of 180 operated larvae, 22 females developed. They were mated to or males with the aim of distinguishing the progeny of the implanted ovary from that of a host ovary. One of the 22 females gave

"Sex-raio-J" of *D. bifasciata* produces no male offspring. The authors consider that the underlying mechanism of this fact can be understood from the viewpoint of cytoplasmic inheritance, as Magni

progeny comprising 4 or females, 3 or males, and 20 + (SR) females. The results obtained so far offer indirect evidence for the hypothesis of cytoplasmic inheritance in D. bifasciata.

Krivshenko, J. Reverse mutation of the gene white to the normal allele.

$sc^8$  B males the following progeny was obtained: ♀♀  $y\ w^+$  = 41, ♀♀  $y\ w$  = 14, ♂♂  $sc^8$  B = 52; total flies = 107.

Individual test crosses of red-eyed females of this progeny (see table) showed that they belonged to two classes: (1) females that produced only red-eyed daughters (3,5,6,11,12), and (2) females that produced red-eyed as well as white-eyed daughters (remainder). The red-eyed females of the first class produced only red-eyed daughters; the red-eyed females of the second class again divided into two classes--females that produced only red-eyed daughters and females that produced both red-eyed and white-eyed daughters. The daughters of white-eyed females in all cases were of the mother's phenotype. The male offspring were of the father's phenotype in all crosses. By crossing white-eyed and red-eyed females (of both classes) with males of  $y$ ,  $EN$  ( $Y^S.X.Y^L$ )/0 stock, the presence of the gene  $bb$ , present in the original  $y\ w\ bb$ = females, was demonstrated.

From the above data it follows that the original red-eyed female carried in one of her X chromosomes the gene white, in the other its normal allele or a suppressor; that is, this female was heterozygous. The origin of this heterozygosity may be due to a reverse, spontaneous mutation of one white gene to its normal allelomorph.

The subsequent appearance in the progeny of the original heterozygous female of the three classes of females (homozygous red, heterozygous white, and homozygous white) is due to crossing over in the attached-X chromosomes. The frequency of the crossing over which took place between the white locus and the centromere of the X (or between the possible suppressor and the centromere) approaches 50%.

#### Results of crosses of yellow, red-eyed females with $y\ w$ males (pair mating)

Cross no.	Phenotype of progeny		♂♂ $y\ w$	Total
	♀♀ $y\ w^+$	♀♀ $y\ w$		
1	32	11	62	105
2	33	5	37	75
3	28	0	40	68
4	23	11	27	61
5	18	0	12	30
6	31	0	36	67
7	35	5	30	70
8	31	9	35	75
9	22	8	35	65
10	7	5	20	42
11	30	0	41	71
12	17	0	25	42

Kuroda, Y., and Tamura, S.  
Effect of metamorphic hormone on the growth of melanotic tumors in D. melanogaster in vitro.

inserted in tissues of the fat body, the hypoderm, the trachea, and the intestine. In the former tumors, no further growth was observed when they were cultured in the synthetic medium (DIS-28, p. 127), whereas the latter tumors showed marked melanotic growth in the surrounding tissue. When cephalic complexes involving the ring glands of mature third-instar larvae were cultured with the tumor tissues, the melanotic growth of tumor tissue was markedly inhibited, as shown in the following table.

Numbers cultured	Melanotic growth of tumors					
	Excellent		Progressive		Slight	
	No.	%	No.	%	No.	%
With the cephalic complexes.	23		2	8.7	5	21.7
Control	42		7	16.7	30	71.4
					16	69.6
					5	11.9

$$(N = 2, \chi^2 = 22.789, P < 0.01)$$

This phenomenon seems to be caused by the metamorphic hormone secreted from the ring gland.

Kuroda, Y., and Tamura, S.  
Effect of PTC (phenylthiocarbamide) on the growth of tumors in D. melanogaster in vitro.

PTC is known to inhibit melanin formation in various animal tissues. Melanotic tumors in the hindgut of mature third-instar larvae were cultured in vitro by the same procedures described in the preceding note, to investigate the effect of PTC upon the growth of melanotic tumors. When 1 mM PTC was added to the synthetic medium, the growth of melanotic tumors was observed to be inhibited almost completely. The results are shown in the following table.

Numbers cultured	Melanotic growth of tumors						
	Excellent		Progressive		Slight		
	No.	%	No.	%	No.	%	
Experiment	43	0	0	4	9.3	39	90.7
Control	42	7	16.7	30	71.4	5	11.9

$$(N = 2, \chi^2 = 53.157, P < 0.01)$$

Kuroda, Y., and Yamaguchi, K.  
Effects of the cephalic complexes on the eye discs of D. melanogaster in vitro.

Eye-antennal discs from mature third-instar larvae (95 hours after hatching at 25° C) of D. melanogaster, which had grown under sterile conditions, were cultured in vitro in the synthetic medium which was described in DIS-28 (p. 127). In comparison with the culturing of the eye-antennal discs alone, the culture of the eye-antennal discs together with

Eye-antennal discs from mature third-instar larvae (95 hours after hatching at 25° C) of D. melanogaster, which had grown under sterile conditions, were cultured in vitro in the synthetic medium which was

cephalic complexes involving the brain hemispheres, the ventral ganglion, and the ring gland showed more pronounced growth and differentiation of the eye discs. The cephalic complexes of a wild strain (Oregon) were found to have the effect of promoting growth and differentiation of Oregon-type eye discs in Bar larvae as well as in Oregon larvae. The cephalic complexes of Bar larvae had the effect of promoting the development of Bar-type eye discs in Oregon larvae as well as in Bar larvae. Those facts suggest that the genic action appears first on the brain and the ring gland in the cephalic complex, which then influence the growth and differentiation of the eye disc.

Lewis, E. B. The nonautonomy of sable in gynandromorphs.

Gynandromorphs produced from the mating of claret nondisjunctional (cand; description in *Genetics* 37: 600-601) females

with  $sn^3$  s males have shown that sable is nonautonomous.

Lindsley, D. L. Heterochromatic exchange between a reversed acrocentric compound X chromosome and the Y chromosome.

Detachment products derived from exchange between acrocentric compound X chromosomes (e.g., double X's) and the Y chromosome provide unambiguous information as to which arm of the Y is involved in the

exchange. If we call the arm involved in the exchange the synaptic arm ( $y_{syn}$ ) and the other arm the asynaptic arm ( $y_{as}$ ), it can be seen from a simple line drawing that detachments which carry the distal X of the compound will always carry  $y_{as}$  whereas detachments which carry the proximal X of the compound will always carry  $y_{syn}$ .

The present experiment involves the use of a reversed acrocentric in which the proximal X is  $In(1)sc^8$  with the distal uninverted  $y^+$   $ac^+$  region replaced by the distal X which is in normal sequence and bears the markers  $y$   $ac$   $sc$   $pn$ ; a normal Y was used. Females of the constitution RA/Y were crossed individually to  $In(1)EN$   $y$   $f$   $B$ / $sc^8$ .Y males. Among detachments that could be tested for the presence of a Y arm, 60 involved the proximal X and only 5 involved the distal X; the reason for such a great discrepancy is not understood. Among the detachments carrying the proximal X,  $y^S$  could be demonstrated on 19,  $y^L$  on 2, and no Y arm on 39. Among the detachments carrying the distal X,  $y^S$  could be demonstrated on 1 and  $y^L$  on 4. Thus in 23 of 26 cases in which the Y was involved in detachment of the reversed acrocentric,  $y^S$  was the synaptic arm.

In addition to the detachments that could be tested, there were 23 recovered in  $F_1$  females which were either lethal or sterile as  $F_2$  males with a complete Y. The detachment event studied appears to be meiotic since the number of females yielding more than one detachment is less, if anything, than expectation on the basis of independence of detachments.

Makino, S., Momma, E.,  
Takada, H., and Wakahama, K.  
Drosophilidae collected in  
Hokkaido.

During a period ranging from May, 1951 to October, 1954, a distribution survey of Drosophilidae was carried out in Hokkaido. Most of the flies were collected by the use of traps baited with fermenting

banana, and a considerable number of specimens were captured by sweeping. The results are shown in the following table. The species marked with \* and \*\* will in the near future be reported as new species or varieties by Dr. T. Okada of Tokyo Metropolitan University.

Species	Specimens obtained	Species	Specimens obtained
<i>Acletoxenus</i> sp.	5	<i>D. melanogaster</i>	371
<i>Amiota variegata</i>	4	<i>D. nipponica</i>	951
<i>A. sp.</i>	21	<i>D. rufa</i>	72
<i>Cacoxenus</i> sp.	8	<i>D. suzukii</i>	125
<i>Chymomyza costata</i>	13	<i>D. bifasciata</i>	1657
<i>Gitona</i> sp.	4	<i>D. helvetica</i>	6
<i>Leucophenga angusta</i> *	11	<i>D. nigromaculata</i>	5401
<i>L. maculata</i>	55	<i>D. transversa</i> var. <i>brachynephros</i> **	1247
<i>L. magnipalpis</i>	1	<i>D. transversa</i> var. <i>rectangularis</i> **	13
<i>L. ornatipennis</i>	2	<i>D. kuntzei</i>	8
<i>Mycodrosophila japonica</i> *	104	<i>D. littoralis</i>	8
<i>M. sp.</i>	37	<i>D. virilis</i>	103
<i>Scaptomyza apicalis</i>	396	<i>D. testacea</i>	807
<i>S. disticha</i>	1362	<i>D. funebris</i>	323
<i>S. graminum</i>	235	<i>D. hydei</i>	18
<i>S. unipunctum</i>	70	<i>D. lacertosa</i> *	337
<i>S. sp.</i>	2	<i>D. moriwakii</i> *	60
<i>Drosophila alboralis</i>	39	<i>D. sp. (robusta-like)</i>	17
<i>D. histriooides</i> *	112	<i>D. sordidula</i>	276
<i>D. nokogiri</i> *	15	<i>D. sp. (grandis-like)</i>	24
<i>D. sexvittata</i> *	444	<i>D. immigrans</i>	807
<i>D. trivittata</i>	370	<i>D. melanissima</i>	39
<i>D. (Hirtodrosophila) sp.</i>	68	<i>D. makinoi</i> *	3
<i>D. coracina</i>	66	<i>D. histrio</i>	120
<i>D. busckii</i>	263	<i>D. sternopleuralis</i> *	36
<i>D. auraria</i>	5997	<i>D. sp. (indeterminable)</i>	49
<i>D. lutea</i>	80		
<i>D. magnipectinata</i> *	62		
		Total	22,724

Mather, W. B. Cytological evolution of eastern Queensland Drosophilae.

particular reference to the genus *Drosophila*, is discussed. The chromosome's of six species of the *pholadoris* subgenus (*D. cancellata* Mather, *D. enigma* Mall., *D. lativittata* Mall., *D. opaca* Mather, *D. maculosa* Mather, *D. levis* Mather), three species of the *Sophophora* subgenus (*D. serrata* Mall., *D. takahashii* Sturt, and *D. dispar* Mather), and one species of the *Drosophila* subgenus (*D. versicolor* Mather) are described and illustrated. The cytological evolution of these species is interpreted in terms of fusions, inversions, and addition of heterochromatin. The cytological picture of the species groups to which the Australian species belong is examined in relation to geographical distribution, and it is shown that these data are helpful in establishing the phylogeny of the *Pholadoris* subgenus.

Mather, W. B. Hybridization of Eastern Queensland Drosophilae.

(The following is the summary of a paper in press with the Australian Journal of Zoology.) The value of cytological methods in evolutionary studies, with

(The following is the summary of a paper to be published in the Australian Journal at an early date.) Hybridization tests have shown that the four Australian

morphospecies of the *Pholadoris* subgenus (*D. opaca* Mather, *D. cancellata* Mather, *D. lativittata* Mall., and *D. enigma* Mall.) are biospecies, but that the following combinations yield adult offspring--♂ *D. opaca* x ♀ *D. enigma* and ♂ *D. cancellata* x ♀ *D. lativittata*--and that "gene exchange" is possible between *D. cancellata* and *D. opaca*. Sexual isolation, hybrid inviability, and hybrid sterility play a part in keeping the species distinct from one another.

The results of the hybridization tests support the hypothesis that the species should be placed in one species group (the *coracina*), as was previously done on the basis of morphological criteria. Concerning the three main morphological characters used for separating these species, viz., thoracic, abdominal, and wing markings, those of *D. opaca* are dominant to those of the other three species and *D. lativittata* characters dominant to those of *D. cancellata*. These character differences between at least *D. opaca* and *D. cancellata* seem to be controlled by one sex-linked gene.

Mather, W. B. Seasonal changes in a natural population of Eastern Queensland Drosophilae.

(The following is the summary of a paper in press with the Australian Journal of Zoology.) The quantitative variations of

sixteen species of *Drosophila* attracted to banana bait at a single station over a period of fifteen months have been assessed. There are two abundant "winter" species; other species fall into "common" and "rare" categories and flourish in either the autumn or the spring. Peaks in the numbers of each species correspond either to the period of greatest rainfall or to the period of rising temperatures. Results have been compared with a similar survey at Aldrich Farm, Texas, U.S.A.

Mattoni, R. H. T., and Tinderholt, Victor. The determination of digenic factors in natural populations.

In recent years hidden genetic variability in natural populations has been extensively studied. Depending on the species of *Drosophila* and the chromosome used, various modifications of the C1B

technique have been employed to make chromosomes homozygous. Thus many types of viability modifiers, including lethals, and visibles have been revealed. We have recently employed a technique enabling us to test two chromosomes, the second and the third, simultaneously in *D. pseudoobscura*. (See crosses on p. 137.)

Thus, the fulfillment of the ratio 4 Ba L : 2 Ba : 2 L : 1 + indicates that the tested chromosomes from the original wild male carried no lethal factors. Other expected ratios are as follows: a 4 Ba L : 2 Ba : 0 L : 0 + indicates a monogenic (single Mendelian) lethal on the second chromosome, a 4 Ba L : 0 Ba : 2 Lb : 0 + indicates a monogenic lethal on the third, and a progeny from a testcross yielding all Ba L flies indicates the presence of two independent monogenic lethals, one on the second and the other on the third chromosome.

One other ratio, however, was hypothesized. This is the absence of only the + ; + class; in other words, a ratio of 4 Ba L : 2 Ba : 2 L : 0 +. Such a situation would indicate the presence of two complementary factors producing lethality when they occur as double recessives. We refer to these as digenic lethals. This would represent the simplest type of polygenic factors.

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### brother-sister mating

$\frac{\text{Ba gl}}{\text{II}} ; \frac{\text{or L}}{\text{III}}$	$\frac{\text{Ba gl}}{\text{II}} ; \frac{+}{\text{III}}$	$\frac{\text{III}}{+}$	$\frac{\text{II}}{\text{II}} ; \frac{\text{or L}}{\text{III}}$	$\frac{\text{II}}{\text{II}} ; \frac{+}{\text{III}}$	$\frac{\text{II}}{+}$
$+$	$+$	$+$	$+$	$+$	$+$
4 Ba L	:	2 Ba	:	2 Lb	:

Visible factors would also be detected in the same manner. Our present results, as yet incomplete, have been obtained from a collection of wild males from Vandeventer Flat in the San Jacinto Mountains. These are tabulated as follows. Further work is in progress on all these strains.

Undetermined	41
Normals	16
II chromosome monogenic lethals	9
III chromosome monogenic lethals	11
II and III chromosome monogenic lethals	4
Probable digenic lethals	2
Probable su L (II chromosome)	1
II chromosome visibles	1
III chromosome visibles	0
Total	86

Meyer, Helen U. Cross reactions of dumpy-comma<sup>2</sup> with members of the dumpy series.

Dumpy-comma<sup>2</sup> (dp<sup>cm2</sup>), though found in ultra-violet-irradiated material, is very likely of spontaneous origin, since all offspring of a treated male heterozygous for a second chromosome marked by bw sp also contained

this mutation; it probably arose in the previous generation; the alternative possibility, that all offspring were derived from the same primordial germ cell, in which this mutation was induced in the chromosome marked by bw sp, is highly unlikely in this case, which belonged to a group that had received only a low dose of ultraviolet.

The mutant was found through its being lethal in compound with  $dp^T$  of Morgan; and when it proved to be nonlethal when homozygous, contrary to expectation, a number of crosses to other  $dp$  alleles, or pseudoalleles, was made to learn more about their various cross reactions with this near-normal mutant. The results are tabulated below; the designation of the different grades of intensity for the various characteristics takes into account both the frequency and the intensity of its expression.

Comparison of Compounds of  $dp^{cm2}$  with Various Other  $dp$  Mutations

Genotype of compound	Phenotypic characteristics, with degree of intensity						
	Thorax		Wings		Deformed legs	Lethal	
	Commas	Vortices	Oblique	Blistered			
$dp^{cm2}/dp^{cm2}$	2	0	0.1	0.5	0	0	
$dp^{02}/dp^{02}$	0	0	2	1	0	0	
$dp^{02}/dp^{cm2}$	0	0	1	0	0	0	
$dp^{v2}/dp^{v2}$	0	2	0	0	0	0	
$dp^{v2}/dp^{cm2}$	0	1	0	0	0	0	
$dp/dp$	2	2	2	1	0	0	
$dp/dp^{cm2}$	3	1	0.1	0	0	0	
$dp^{tx}/dp$	2	2	0	2	0	0	
$dp^{tx}/dp^{cm2}$	3	2	0*	2	0	0	
$dp^{txI}/dp$	2	3	0	2	0	0	
$dp^{txI}/dp^{cm2}$	2	0	0*	3	0	0	
$dp^{T51b}/dp$	0	0.1	3	1	1	0	
$dp^{T51b}/dp^{cm2}$	0.1	0	2	2	0	0	
$dp^T/dp$	3	2	3	1	2	0	
$dp^T/dp^{cm2}$	3	0.5	3	1	2	2**	
$dp^{T54d}/dp$	1	1	2	1	1	0	
$dp^{T54d}/dp^{cm2}$	3	1	2	0.5	2	2**	
$dp^{T55c}/dp$	2	1	3	3	2	0	
$dp^{T55c}/dp^{cm2}$	?	?	?	?	?	3	

\* Narrow wings.

\*\* Sublethal at high temperature.

The cross reactions which have so far been studied are listed in the table, where they are compared with the phenotypic characteristics of the homozygotes, or, in the case of the lethal alleles, with those of the compound with  $dp$ .

It becomes apparent that  $dp^{cm2}$ , although quite similar in its action to the normal gene, is a useful tool for revealing potentialities of each particular dumpy allele. It promotes expression of the comma effect and of the blistered-wing effect. It also promotes (as compared with the action of the

normal allele), though to a much lesser degree, the expression of "oblique" (truncated) wing, of vortices, and of the lethal of truncates ( $dp^T$ ,  $dp^{T54d}$ ,  $dp^{T55c}$ ). This promoting action is exerted despite the fact that  $dp^{cm2}$  itself does not show these effects when homozygous. It is curious that the order of intensification of comma and of vortex in compounds with other members is at several points different from the order shown by these members when homozygous or in their compounds with dumpy. Strangely,  $dp^{cm2}$  does not promote--at least not to a conspicuous degree--the lethal effect of thoraxates ( $dptx$ ,  $dptxI$ ) or of lopped ( $dp^{T51b}$ ). This ability of  $dp^{cm2}$  to differentiate between the lethals of the different lethal dumpy alleles might prove useful in the further investigation of the pseudoalleles at the dumpy locus.

(This work has been supported by a grant received for work of H. J. Muller and associates from the American Cancer Society, on recommendation of the Committee on Growth of the National Research Council.)

Milani, R. Effect of natural selection on the full expression of the gene counter-coiled (cc).

of the body. These males are sterile as the result of a mechanical impediment to copulation. During stockkeeping a steady, regular decrease of such males has been observed, from about 75% to about 30%.  $F_2$  segregants from crosses show 100% abnormal specimens. Progressive substitution of the residual genotype of cc flies from a stock at the 30% level produced a progressive increase in the frequency of abnormals.

The gene cc (DIS-28) reverses the coiling direction of the male genitalia. Many specimens do not develop perfectly, and the plane of symmetry of the hypopygium does not coincide with the sagittal plane

Milani, R. Genes affecting tergal polymorphism of the housefly.

phenotypes are: (a) females without a central stripe and males with a thin black stripe; (b) females and males with a thin black stripe; (c) females and males self black; (d) black females and striped males. The last phenotype is due to a single autosomal recessive gene, which acts as a sex-limited inhibitor of black when black is homozygous; its presence can be detected in both sexes when black and its inhibitor are both heterozygous.

The Italian populations of houseflies are polymorphic for tergal pigmentation. Some genes have been isolated which control the pigmentation pattern. Their corresponding

Milani, R. Inheritance of Musca domestica mutants.

These characters are monofactorial recessives. No indications of criss-cross inheritance have been found; disturbances in the  $F_2$  recombination classes have been observed for the genes brown body (analogous or homologous to  $y$  in *Drosophila*) and divergent wings. The double-recessive class is much more affected when the two markers have been contributed one by each parent in the original cross than in the reciprocal case (when both recessives come from the same fly). Backcross tests have not yet been made. Gynandromorphs showing mosaicism for the two genes have been found, with somatic distribution of the two characters in accordance with the type of the original cross: joined when they came from the same  $P$  fly, opposite when they came from the two  $P$  flies. These facts suggest the hypothesis of linkage. If this should be proved, crossing over must happen in the male housefly.

The mutant strains brown body, short wings, counter-coiled, divergent wings, and clipped have been genetically tested. All

Milkman, Roger. Interaction of various "crossveinless" genes in D. Melanogaster.

Although  $cv/+$   $cv-c/+$ ;  $cv/+$   $cv-d/+$ ; and  $cv-c/+$   $cv-d/+$  D. melanogaster all have normal posterior crossveins at  $25^\circ C$ , each of these genes does interact with a

polygenic "crossveinless" system (cve) similar to systems reported previously by Waddington and, in DIS-28, by Bateman.  $F_1$ 's from the following crosses were compared as to average degree of crossvein absence (each fly being rated from 0, wild type, to 12, no posterior crossvein at all): cve x Ore-R, cve x cv, cve x cv-c and cve x cv-d. Results show a clear interaction in the last three crosses, though present data are very scanty for cve x cv-d. Waddington has reported interaction between his cvl and cv, which is higher than that reported here.

A strain of det from California Institute of Technology was also tested. This strain is not true breeding; the character interacts with cve and cv-c. It responds to selection like a polygenic system and therefore must consist of more than the single factor originally proposed. Since det behaves like cve, and produces an identical range of phenotypes, and since in selection for factors interfering with posterior crossvein formation it would be impossible to discriminate between det and cve, there is no reason to assume that cve and det are not identical, or at least components of a more extensive polygenic system.

The possibility that cve consists at least in part of alleles at the cv, cv-c, and cv-d loci, as well as at the det locus listed in Bridges and Brehme, is now being investigated.

Cross	Females			Males		
	Total	Crossveinless	Rating	Total	Crossveinless	Rating
cve x cve	35	35	9.2	30	30	8.1
cve x Ore-R	219	27	0.23	180	18	0.17
Ore-R x cve	186	37	0.33	188	1	0.005
cve x cv	279	88	0.94	(256	25	0.17)
cv x cve	122	45	0.89	(100	100	12
cve x cv-c	295	257	4.7	288	264	6.2
cv-c x cve	126	126	7.3	157	122	3.5
cve x cv-d	7	4	2.6	2	1	2
cv-d x cve	28	16	1.3	24	0	0
cve x det	24	23	5.0	27	23	6.2
det x cve	22	14	2.4	20	11	1.0
det x cv-c	164	88	1.5	167	16	0.15
det x det	146	116	2.7	163	45	1.2

Miller, D. D. A study of sexually isolated eastern and western D. athabasca.

Eastern (Michigan) and western (mainly Wyoming, but with some North Dakota and western Ontario) strains of D. athabasca were found to be sexually isolated. Ten-day, no-choice cohabitations led to 69% (133/193) inseminations of eastern

day, no-choice cohabitations led to 69% (133/193) inseminations of eastern

females by their own males, and 69% (147/212) inseminations of western females by own males; but such conditions led to no inseminations (0/294) of eastern females by western males, and less than 1% (2/277) inseminations of western females by eastern males. No reliable morphological or cytological (metaphase chromosomes) differences could be found between the eastern and western D. athabasca of these strains. Observations of intrastrain mating behavior, however, showed that the duration of copulation was always less in the eastern strains than in the western ones, as is shown in the following table of first copulation times of week-old adults:

Temperature	Western <u>D. athabasca</u>	Eastern <u>D. athabasca</u>
84° F	5'19", 5'34", 5'54"	1'34", 1'34"
79° F	7'48"	
78° F	4'37", 4'55", 7'16" 7'56", 9'54", 11'3"	1'18"
76° F	6'50", 7'27", 8'25"	1'47", 1'59"
75° F	3'57", 6'32", 7'37"	1'10", 1'33", 1'35", 1'40", 2'0"
74° F	4'17", 6'0", 11'36", 15'29"	1'6", 1'9", 1'34", 1'41", 1'56"
73° F	4'58", 6'27"	1'21", 1'33"
Range:	3'57" - 15'29"	1'6" - 2'0"

The range of copulation times of the observed Michigan strains covers that of previously studied strains of D. athabasca from Cold Spring Harbor, N.Y. and Princeton, N.J. (1'12" - 1'48", Miller, DIS-25, 1951), whereas that of the current western strains includes the range of copulation times reported by Spieth (1952) for a Wyoming strain of this species (7'10" - 10' 5"). Novitski (1946) reported no sexual isolation between the eastern and western strains of D. athabasca available to him; however, he had no strains from Michigan. Research in progress involves testing the mating reactions of newly established D. athabasca strains from Cold Spring Harbor, New York.

Momma, E. Some predominant species of *Drosophila* in Hokkaido.

In level lands, D. auraria, D. nigromaculata, and D. transversa are most prevalent in collections and show the most extensive distribution. D. auraria occurs as a dense population in southern districts, whereas D. nigromaculata is dense in northern parts of Hokkaido. In highland areas, D. bifasciata, D. testacea, and D. nigromaculata are the most dominant drosophilids. D. nigromaculata feeds on various kinds of fungi and grasses as well as fermenting fruit. Specimens of D. transversa have been observed on several kinds of fungi and on fermenting gruit. Very few flies of other species have been found on these grasses or fungi. These other species have been captured in traps baited with fermenting banana.

Momma, E., and Wakahama, K.  
Seasonal behavior of  
Drosophilidae at Sapporo.

Field observations were carried out in the University Botanical Garden at Sapporo from May to October, 1954, with special regard to the seasonal variation in

relative frequencies of drosophilid flies. During the snowfall season, extending from November to March, flies were not attracted to the baits. Collections were made continuously for three days in the last third of every month, using traps baited with fermenting banana. The results are summarized below.

Species	May	June	July	Aug.	Sept.	Oct.	Total specimens
<i>Leucophenga maculata</i>	-	-	2	-	1	-	3
<i>Scaptomyza graminum</i>	-	1	12	1	1	-	15
<i>Drosophila histrionoides</i>	-	1	-	1	-	-	2
<i>D. coracina</i>	-	1	-	-	-	-	1
<i>D. auraria</i>	-	13	93	350	17	1	474
<i>D. lutea</i>	-	-	-	8	1	-	9
<i>D. magnipectinata</i> *	4	-	-	1	2	-	7
<i>D. melanogaster</i>	-	-	1	56	4	-	61
<i>D. nipponica</i>	-	4	5	1	1	3	14
<i>D. rufa</i>	1	6	1	-	2	1	11
<i>D. suzukii</i>	-	-	-	1	1	-	2
<i>D. bifasciata</i>	-	2	1	18	-	-	21
<i>D. nigromaculata</i>	160	100	636	86	191	36	1209
<i>D. transversa</i>							
var. <i>brachynephros</i> *	3	60	27	153	49	5	297
<i>D. virilis</i>	-	-	-	6	-	-	6
<i>D. littoralis</i>	-	-	-	3	-	-	3
<i>D. testacea</i>	8	4	7	58	-	-	77
<i>D. funebris</i>	-	-	-	2	-	-	2
<i>D. hydei</i>	-	7	3	-	-	-	10
<i>D. lacertosa</i> *	-	2	-	23	8	-	33
<i>D. sordidula</i>	-	-	-	3	-	1	4
<i>D. immigrans</i>	-	1	-	8	13	1	23
<i>D. histrionoides</i>	-	-	-	8	-	-	8
<i>D. sp.</i>	-	1	-	1	1	-	3
Total	176	203	788	788	292	48	2295
Mean temperature (C)	10.6	13.8	17.7	20.6	17.9	9.3	
Mean humidity (%)	69.1	72.5	78.6	84.8	78.9	72.7	

\* Manuscript name

Moree, R. The question of generation length in population models of *D. melanogaster*.

In calculating theoretical selection curves the unit of time employed is one generation, whereas in plotting experimental selection curves from population data the unit of time is one day. These curves can be compared satisfactorily only if they can be converted to a common time scale, expressed as, say, days per generation. That some difficulty has been experienced in this is shown by the fact that different authors, using various bases of determination, have taken the length of a generation to be as follows:

	20° C	25° C
Morgan, Bridges, and Sturtevant. 1925. The Genetics of <i>Drosophila</i> . Bibl. Genet. 2: 1-262. (p.15).....	14.5	9.2
Demerec and Kaufmann. 1945. <i>Drosophila Guide</i> . 4 ed.		
Carnegie Inst. of Wash. 1-44. (p.3).....	14.3	9.2

		20° C	25° C
Reed and Reed. 1950. Evolution 4: 34-42. (p.37) .....	.....	21.0	(14.0)
Wallace. 1950. Evolution 4: 172-174. (p.172) .....	.....	(21.0)	14.0
Ludwin. 1951. Evolution 5: 231-242. (p.233) .....	.....	30.0	(20.0)
Merrell. 1953. Evolution 7: 287-296. (p.292) .....	.....	24.0	(16.0)
Wallace. 1953. Genetics 38: 456-470. (p.460) .....	.....	14.0	
Prout. 1954. Genetics 39: 529-544. (p.530) .....	.....	14.0	
Erk. 1955. Genetics 40: 331-342. (p.339) .....	.....	12 to 15	

(The times in the first two entries represent the average minimum time within which a life-cycle can be completed, rather than the generation time, which is of course slightly longer. Figures in parentheses are not those of the authors quoted, but are computed on the assumption that at 20° C the length of generation is approximately 1.5 times that at 25° C.)

Any or all of several different factors might be responsible for the reported variations in length of generation, such as different conditions of experimentation, genetical differences among the strains of flies used, or an unknown degree of crowding in the population model which might increase the generation time. But the chief difficulty in making the necessary time scale conversion results from the overlapping of generations in the population model, some aspects of which have been discussed by Merrell (Evolution 7, p. 292, and in private conversation). The condition of overlapping, which also decreases such selective effects as may be present, introduces a difficulty in the very concept of what should be taken to constitute generation length in a continuous population. An additional difficulty, of course, would be the application of the concept to the population model, whatever the concept might be. As far as I am aware, these problems are essentially unsolved at the present time.

Morita, T., and Oshima, C.  
Phenol-reagent-positive substances extracted from larvae and puparia of D. virilis and D. americana.

The amounts of tyrosine, xanthine, and uric acid which were contained in third-instar larvae and puparia of wild strains of both species and a mutant "ebony" of D. virilis were compared by the following method. Dry-material (50 mg) was extracted with 80% ethanol and centrifuged. The supernatant was concentrated and the residue was dissolved in 5 ml of 1/10 M sodium citrate buffer solution (pH 4.5) containing benzylalcohol (2.5% v/v). Two ml of the solution were placed on a column (16 cm) of IR-112 (Na-type resin). The effluent with the sodium citrate buffer solution was fractionated by an automatic fraction collector (1.5 ml per fraction). Each fraction was divided into two parts. One part was tested by Folin's phenol reagent, another by uric acid reagent. The amounts of tyrosine, xanthine, and uric acid were determined colorimetrically. It was found that the expected amounts of tyrosine and xanthine were extracted equally from larvae of the three strains, but uric acid could not be detected.

On the other hand, uric acid was detected in the puparia of the three strains. D. americana, having a red puparium, showed the highest content, and the mutant ebony, having a black puparium, the lowest content.

Moriwaki, D., Shirai, M., and Yoshida, Y. H. Balanced polymorphism attained in some experimental populations of D. ananassae.

In D. ananassae, an inversion in the left arm of the second chromosome, InIII, has long been known. Accordingly, there are two different types of gene arrangements,

the reverse of each other. Upon examination of one hundred samples from a wild stock of a Hawaii strain in 1951, we found that it carried these two types, A and B, in a zygotic ratio of: AA, 36%; AB, 52%; BB, 12%. Two stocks,  $H^A$  and  $H^B$ , were made from the Hawaii strain by selecting a pair culture that carried only homozygotes of A or B. Similarly, an  $M^A$  stock, homozygous for A, was separated from a Mexico strain. Experiments with these types, using fifteen population cages, showed that when one type was cultured together with another type having the reverse arrangement, in a definite initial ratio, they nearly always attained the same equilibrium values. This was probably caused by "heterosis," the structural heterozygotes (AB) being more adaptive than the homozygotes (AA and BB). Recently, however, we have obtained in two cages another equilibrium value lower than the previous average. Though similarly planned, the experiments with these two cages had a common difference from the previous fifteen cages. The flies initially introduced into the cages were prepared from new stocks,  $H^{54A}$  and  $H^{54B}$ . These were selected in December, 1954, from the Hawaii strain, which had been kept as a stock since 1951 when  $H^A$  and  $H^B$  were separated from it. During this period (1951-1954) the zygotic frequencies of A and B seem to have changed considerably in the Hawaii stock, since the present ratio is AA, 19%, AB, 60%, and BB, 21%, showing an increase of B at the expense of A. We may attribute the cause of the recent lower equilibrium value, that is, the decrease in A, to genetic changes that arose in the Hawaii stock. It goes without saying that the stocks separated earlier,  $H^A$  and  $H^B$ , have also passed through five years, but the genetic complexes contained in the left arm of the second chromosome of these stocks seem to have been left almost unchanged.

Although the equilibrium values are not necessarily constant, it can be said that balanced polymorphism was established owing to "heterosis." In order to discover some of the reasons for the over-all adaptive advantage of the AB heterozygotes, physiological comparisons were made among the three karyotypes. The traits chosen for study were fecundity, hatchability, rate of development, and sexual activity. In all cases except hatchability, adaptive differences were shown to exist. The data on egg-laying capacity (fecundity) indicate that the AB heterozygotes are superior to both homozygotes, AA and BB. Computing from daily records of pupation and emergence numbers, we can say that AB shows a significantly shorter developmental period than AA, and BB is the slowest. By means of a multiple-choice method it has been known that AB flies of both sexes have a higher crossability than either of the homozygotes, whereas BB has the lowest. In addition, the development of the heterozygotes seems to be better buffered against environmental changes than that of either homozygote, indicating a homeostatic buffering.

Morpurgo, G., and Nicoletti, B.  
Experiments of selective mating in evaluation of gene frequencies in D. melanogaster.

experiments with nonisogenic and isogenic stocks of Oregon and white show a different and more complicated situation. With both isogenic and nonisogenic stocks, we have found selective mating under different experimental conditions.

Strong selective mating was found when two males of different phenotypes were put together with only one female of any genotype. On the contrary, we were not able to find evidence of selective mating in populations in which the sex ratio was 1:1.

Some workers (Reed and Reed; Merrel) think they have found evidence that selective mating is mainly responsible for the advantage of wild type in competition with sex-linked mutants. Our

Moreover, the decrease in white gene frequencies, in populations having the possibility of selective mating, is quite similar to that in others where this possibility is not present. It is also possible to obtain an enormous increase in selection against *w* by simply increasing competition at the larval stage; in default of competition, on the contrary, the expected and observed ratios are extremely similar, irrespective of the possibility of selective mating. Full data and statistical evaluation are in press.

Therefore, according to our data, the hypothesis that nonrandom mating is a primary factor of selection in artificial populations seems not entirely correct. In these populations, in effect, larval competition, crowding, differential developmental speed, double insemination, and so forth are very important factors, which, together with the possibility of selective mating, determine the fitness of individuals having a particular genotype in relation to others having a different genotype. We think, moreover, that it is possible to give a different interpretation to the data of the above-mentioned authors, so that they support our hypothesis.

Morpurgo, G., Nicoletti, B., and Solima, A. Observations on sex ratio in *D. melanogaster*.

Several thousand eggs were collected from many stocks and crosses of *D. melanogaster*, and put together in vials (3.3 cm internal diameter), 50 to a vial. Competition at the larval stage was very low: on the average, 78.84% of the total number of eggs reached the adult stage. Lethality in the embryonic stage showed high variability among the different stocks, crosses, and generations. All emerging flies were scored for sex and the observed sex ratios are reported in the table. The same table also reports data on embryonic and larval lethality. The sex ratio was always close to 1:1, and often there was a slight but generally not significant excess of males. Since in earlier work we found evidence that in larval competition more males than females die, it seems reasonable to assume that the primary sex ratio in *D. melanogaster* shows a tendency toward an excess of males.

<u>Crosses</u>	<u>No. of eggs</u>	<u>% un-hatched</u>	<u>♀ emerged</u>	<u>♂ emerged</u>	<u>% of eggs reaching adult stage</u>
♀ Or x ♂ Or	2000	8.8	711	688	69.9
w/w x w/y	1000	6.9	410	424	83.4
vg/vg x vg/vg	2000	20.0	808	771	78.9
ssa/ssa x ssa/ssa	1500	15.2	608	635	82.8
vg/vg x +/+	1000	14.4	422	375	79.7
+/+ x vg/vg	1000	23.6	300	289	58.9
vg/+ x vg/+	1000	1.30	466	462	92.8
vg/+ x vg/vg	1000	0.80	435	469	90.4
vg/vg x vg/+	1000	14.70	375	385	76.0
+/+ x vg/+	1000	3.90	407	410	81.7
vg/+ x +/+	1000	4.90	414	380	79.4
vg/+ x ssa/+	1000	2.00	420	482	90.2
ssa/+ x vg/+	1000	4.30	389	457	84.6
ssa/+ x ssa/ssa	1000	21.56	338	361	69.9
+/+ x ssa/+	1000	5.10	381	407	78.8
ssa/ssa x ssa/+	650	20.31	205	234	67.5
ssa/+ x ssa/+	1000	2.80	414	366	78.0
<b>Totals</b>	<b>19,150</b>		<b>7503</b>	<b>7595</b>	<b>78.84</b>

Muller, H. J. Correction of localization of crs and breaks of  $Y:bw^+$ .

It was correctly stated in DIS-16, p. 64, that the left and right breaks of the portion of chromosome II inserted into the  $Y$  (long arm) in the  $Y:bw^+$  chromosome

discovered by Dempster, were both somewhat to the right of the corresponding breaks of  $P^1$  (the Pale insertion). However, in DIS-25, p. 119, the latter breaks were through a slip of writing stated to be "to the right" of the former, and crs (the male-sterile associated with "cream-underscored" of Bridges) was then, in accordance with this error, stated (on p. 33) to be between the two right breaks instead of between the left ones. To recapitulate the facts of the case: the two insertions are for the most part overlapping, both of them covering  $bw$ ,  $mr$ , and  $sp$ , but only that of  $P^1$  covering  $px$ , crs, and (as I now find)  $M(2)1^2$ , while the insertion of  $Y:bw^+$ , unlike  $P^1$ , covers the recessive lethal effect of the homozygous Pale transposition, a fact showing this lethal to be associated with the right-hand second-chromosome break of that transposition, and showing also that the  $Y:bw^+$  insertion extends further rightwards than  $P^1$ . Because of  $M(2)1^2$  (and possibly other Minutes) being in the region between the left-hand breaks of the two transpositions, flies heterozygous for Pale deficiency and supplied with  $Y:bw^+$  but not with  $P^1$ , although viable and fertile, have a Minute-bristle phenotype. The right break of the  $Y:bw^+$  insertion is not far enough to the right to allow this insertion to cover M33a.

Muller, H. J. Improvement of stock "Maxy," for studying mutations at specific loci in the  $X$  of the male.

As reference to our new stock list, section f, will show, the "Maxy" stock has been made more effective by inclusion in the multiple-recessive  $X$  chromosome of the mutant genes  $ec$  and  $odsy$ , the former

known to originate fairly often and the latter of interest because of its pleiotropy or locus "nest," and also by inclusion in that chromosome of the long scute  $Sc$ -scute 8 inversion combination. The simultaneous presence, in the homologous  $X$ , of  $In49$  and  $B^M1$ ,  $In$  results in the virtually total elimination of  $X$ -chromosome crossovers. This in turn made it feasible to remove the dominant marker  $Tu$ , which had hindered the recognition of the wing mutants  $N$ ,  $ct$ , and  $dy$ .

The homologous  $X$  ( $1J1$   $Sc^S1$   $In49$   $m$   $B^M1$ ), which is present also in the great majority of the males, has now been supplied with the marker  $m$ , for the purpose of conspicuously marking and also hindering the breeding of exceptional females homozygous for this chromosome; these are able to live despite their homozygosity in  $1J1$  by virtue of having a  $Y$  of type  $1J1^+Y$ .

By the above combination of means, the stock may be maintained without selection and mass operations with it become feasible for the first time, tests carried out by the Altenburgs having shown that the old "Maxy" stock gave exceptions that were too viable, inconspicuous, numerous, and productive to allow such mass breeding with that stock. It was for this reason that the present stock was constructed. For cases in which it is desired to distinguish the generations, and to distinguish flies arising by nondisjunction of the  $X$ 's in the female parent, males are also obtainable (from our stock designated as "Maxy-no  $odsy$ ") which are like those just described except for the inclusion of  $v$  in addition to  $m$  in their  $X$ . When these are used in alternate generations with the non- $v$  males, vermillion crosscrosses back and forth among the "regular" (disjunctionally produced) offspring between the males and females.

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on recommendation of the Committee on Growth of the National Research Council.)

Muller, H. J. Male-sterility of transformed females despite provision of X:Y balance characteristic of males.

299, 1945) was caused by lack of sufficient doses of the fertility genes of their Y, in relation to their diplo-X condition, flies were made up that contained 2 X's and 2 Y's (in the form of homozygotes for the  $Y^S.X.Y^L$  chromosome constructed by Novitski) and that were at the same time homozygous for tra. These flies, used as males, proved entirely sterile, despite the fact that they behaved in apparently normal fashion in copulation. That no recessive male-sterility gene was present in the third chromosome was proved by the fertility of their brothers that contained only one  $X^S.X.Y^L$  chromosome and were homozygous for tra.

(Work supported by grant from the American Cancer Society on recommendation of the Committee on Growth of the National Research Council.)

Muller, H. J. Testing for third-chromosome mutations by means of crisscrossed lethals.

This technique is similar to that described for the second chromosome in DIS-27 (p. 104-6), except that balanced male-steriles are not available. Suppose that a third chromosome of given composition, present heterozygously in " $P_1$ " males, is to be tested for mutant genes, and that, in order for its presence in the  $F_1$  flies to be distinguishable from that of its homologue, the two homologous third chromosomes of the  $P_1$  males differ in regard to some dominant third-chromosome marker, or in regard to some recessive marker (such as  $ri$ ) that is present in one or both of the third chromosomes of the females with which these  $P_1$  males are crossed for testing. These females are derived from a stock, here termed "III-tester-1," having 4 balanced lethals, together with inversions effectively preventing the survival of crossovers, in the following arrangement:  $ru\ h\ D^3\ ri\ InRC\ e\ 13e/Me, Ins\ Sb^1$ . The lethals here are  $D^3$ ,  $13e$ ,  $Me$ , and  $Sb^1$ .  $F_1$  males are then picked out that show by their marker that they have the paternal third chromosome to be tested. They may contain either maternal III. These males are crossed to females of a stock, here termed "III-tester-2," of the criss-crossed-lethal composition  $Me, InL\ InRC\ e\ 13e/ru\ h\ D\ InsCXF\ Sb$ . The  $F_1$  males derived from any given  $P_1$  male are crossed collectively, that is, in mass culture, if the original mutant-gene composition of the third chromosome of that  $P_1$  male, as present in the fertilized egg from which the male had developed, is to be tested, but are crossed individually, in any desired number of cultures, if the mutations that occurred subsequently, in the course of the  $P_1$  generation itself, are to be studied. The  $F_1$  are discarded before the eclosion of the  $F_2$ .

No  $F_2$  flies survive to the imaginal stage unless they received the paternal third chromosome that is to be tested and a maternal homologue containing the dominant markers  $D$  and  $Sb$ , or  $Me$ , with inversions, except for relatively rare cases (not in themselves a cause of erroneous scoring) representing simultaneous complementary autosomal nondisjunction. The  $F_2$  from each culture can be transferred en masse for interbreeding, virgins being unnecessary. The  $F_3$  are examined for the absence (indicative of a lethal) or

presence (in which case they may be inspected for visible mutations and/or tested for fertility) of flies homozygous for the third chromosome being tested. These flies are recognized by their nonpossession of any of the dominant markers D, Sb, Me, and, in case one or more recessive markers had been supplied to begin with, by the manifestation of these recessives. To avoid errors caused by the rare crossing over between the two inversions of the Me, InL InRCe 13e chromosome, in cases where there was no recessive marker in the right arm of the third chromosome to be tested,  $F_2$  females of the D Sb type could have been picked out, instead of making en masse transfers;  $F_2$  males of the same type may also be preferable to the Me males, especially for establishing balanced stocks of lethal or sterile mutants. For such establishment,  $F_3$  or still later-generation males and virgin females that show D Sb but do not show Me may be mated together.

Both third chromosomes of the " $P_1$ " males are testable if neither has intentionally been supplied with a pre-existing lethal. Moreover, " $P_1$ " females can be tested likewise, provided the production of crossover  $F_1$  can be prevented, as by inversions or c3G. The  $F_1$  flies used can also be females, if those females are chosen for breeding which received from the "III-tester-1" stock the third chromosome which more effectively prevents crossovers from appearing (that containing Me, Ins). Another possible variation is to cross the " $P_1$ " flies to "III-tester-2" and the  $F_1$  to "III-tester-1." In this case the  $F_2$  females which are preferable, because of their more effective prevention of crossovers, are those of Me phenotype.

It is evident that when such an experiment is carried out the cultures showing no mutation provide isogenic stocks that can be used as the start of a new series of tests, since they are free of pre-existing lethals, etc. That is, the testing is in itself the most effective way of getting isogenic stocks of III and thus of preparing for another mutation experiment. However, if these isogenic lines are to begin with homozygous for III they cannot be used for the accumulation of mutations through a series of generations, since natural selection would act in them against deleterious recessives. This objection is avoided if the isogenic lines are balanced (instead of homozygous), by reason of having been provided, in the third chromosome to be tested, with recessive genes that sterilize (but do not kill) both males and females. We have not as yet made up such a combination, but if tra (the former sterilizing the males, the latter the females) would fit the requirement. The resultant accumulation would of course occur, on the average, in approximately half of the generations in females and in the rest in males. For accumulation confined to one sex (males) the reader is referred to Oster's ingenious method described in a parallel article in this issue.

The present scheme (except for its potential accumulation feature) has already been tried out on a considerable scale by Dr. Meyer in combination with the similar crisscross scheme for chromosome II, for the simultaneous detection of mutations in both II and III. The "II-III-tester-1" stock usually employed in these operations has been "CySMs" (designated j46 in DIS-29). It was found better to cross each of the resulting  $F_1$  males, individually, to two kinds of females, representing separate "tester-2" stocks for the second and third chromosomes, respectively, rather than cross them to flies of one joint "II-III-tester-2" stock. This was partly because use of the latter stock led to too much crossing over in chromosome II, because of the simultaneous inversions in III, and partly because it caused too much loss of viability and fertility on the part of the flies capable of breeding at all, and to the appearance of the desired or diagnostic types at frequencies that were too low. The "II-tester-2" stock used is designated in

our DIS-29 list as j55 and the "III-tester-2" as j79. Each of these stocks is provided with a homozygous recessive in that pair of major autosomes which is not being tested by it. Thus, if the cross of the  $F_1$  male to one of the "tester-2" stocks happens to fail, the analysis of the major autosome that was to have been tested thereby can be continued anyway (even though delayed by two generations), by the use of flies derived from the cross with the other "tester-2" stock.

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Muller, H. J., Herskowitz,  
I. H., and Oster, I. I.

Effect of narcosis on X-ray-induced mutations in sperm treated in inseminated females.

The  $CO_2$ . This probably occurred at a stage long before the removal of most of the oxygen. A second group of flies was placed in capsules the ends of which were replaced by dacron netting, to furnish a normal supply of air.

The former group of flies gave frequencies of all types of mutations studied (lethals, translocations, and chromosome losses) that were significantly lower than those given by the group irradiated in air. As expected because of their multibreak nature, translocations were the most influenced in their frequency.

(Work supported by grant from the Atomic Energy Commission, Contract AT (11-1)-195.)

Nolte, D. J. Eye-color polygenes.

During the study of South African strains of D. melanogaster it has become evident that significant differences occur in the quantities of the red and brown eye pigments in strains from different localities, as well as in strains collected during different years in some localities. These increases or decreases are differential for the two pigments, the one pigment increasing or decreasing in amount independently of the other.

It appears that modifying genes occur in this species, and that these genes are active in affecting the final quantitative results of the action of the various oligogenes interacting during the eye-pigmentary processes. In the  $F_1$  of crosses between strains the quantitative effect is intermediate, and in one particular cross these modifying genes appeared to be of a dominant nature with regard to the red-pigment content but of a recessive nature with regard to the brown-pigment content. It appears as if two series of polygenes segregate in some wild populations of D. melanogaster, affecting the red and brown pigments, respectively.

A study, now in progress, of the chromosome races of D. pseudoobscura--viz., Standard, Arrowhead, and Chiricahua--is yielding results comparable to the above.

Nordback, K. Effect of storing spermatozoa in the male on the frequency of X-ray-induced lethals and translocations.

In three experiments, 3-4-day-old Ore-K males were exposed to 3500 or 4000 r. In each experiment, three series were run. Series I consisted of males which immediately after exposure were mated in mass (20 ♂♂ : 30 ♀♀ per bottle in Exp. 1 and 2,

15 ♂♂ : 60 ♀♀ in Exp. 3). After 24 hours, the males were remated to fresh females and left with them for 24 hours. This gave Series II. Series III consisted of males which after exposure were kept without females for 24 hours and were then mated in the same way as the males in the other two series. In all experiments, the females were allowed to lay eggs for one or two more days after removal of the males. The females came from dual-purpose stocks and allowed the testing of sex-linked lethals and translocations between the large autosomes in the same progenies ( $sc^{S1} InS w^a sc^8$ ;  $vg$ ;  $e$  in Exp. 1 and 2;  $y sc^{S1} In49 sc^8$ ;  $bw$ ;  $st$  in Exp. 3). The results are summarized in the table.

Expt.	Series	n	Lethals		Translocations	
			%	n	%	n
1	I	273	14.0	109	13.8	
	II	413	9.5	236	8.1	
	III	375	8.5	159	6.9	
2	I	1022	10.0	733	9.5	
	II	1060	8.5	917	5.8	
	III	1056	8.8	820	6.1	
3	I	278	6.9	283	6.0	
	II	244	3.4	223	4.0	
	III	634	6.7	260	3.5	

Two points are to be noted about these experiments.

1) In all translocation tests and in two of the three lethal tests, Series II and III gave practically the same result. This suggests that the reduction in genetical effect on the second day is due to storing of the irradiated spermatozoa in the male and not to a difference in sensitivity between spermatozoa utilized on the first and second days. This is supported by the finding that the drop in the frequency of translocations from the first to the second day of mating was not greater in Experiment 3, where a superfluity of females had been used, than in the other two experiments with limited opportunity for copulation.

2) In all three experiments the frequencies of both lethals and translocations were reduced by storing--in Experiments 1 and 3 to a similar extent, in Experiment 2 somewhat less for lethals. This suggests that the "recovery" which occurs with storing is not restricted to effects based on breakage and reunion.

When the same kind of experiment was carried out on young males (0-2 days), no effect of storing was found in two repetitions. As regards lethals, this is in agreement with data by Lüning in DIS-28; but in Lüning's experiments storing in young as well as old males reduced the frequencies of hyperploid males and other changes presumed to be intergenic. Several experiments on the influence of storing irradiated sperm in females gave contradictory results; in some of them there was a reduction of effect after storing, but in the majority no such effect was noted.

Okada, T. Intergeneric and intersubgeneric relations in the family Drosophilidae with respect to phallic organs.

From a comparison of about 100 Japanese species of Drosophilidae belonging to 10 genera, with respect to fourteen selected characters of the phallic organs, the following conclusions were reached regarding intergeneric and intersubgeneric relations. In Steganinae, Amiota and Leucophenga are closer to each other than they are to Stegana. In Drosophilinae, Drosophila, Chymomyza, and Liodrosophila are closely related to one another; Scaptomyza most closely resembles Liodrosophila; Mycodrosophila is intimately related to Styloptera (or Dettopsomyia); and Microdrosophila is somewhat apart from any of the foregoing genera. Among genera of different subfamilies, the closest resemblance is seen between Amiota and Drosophila or Chymomyza. The subgenera of the genus Drosophila can be divided into two groups: Drosophila s. str., Hirtodrosophila, and Dorsilopha in one group; and Sophophora and Paradrosophila or Pholadoris in another.

Okada, T. Systematic position of the genus Leucophenga in the family Drosophilidae.

It is proposed that the genus Leucophenga Mik, which has hitherto been placed in the subfamily Drosophilinae, might better be classified as Steganinae, because of the fact that this genus has special morphological features characteristic of the genera of Steganinae (e.g., Stegana Meigen, Amiota Loew). These features are: (1) posterior reclinate orbital bristle no nearer to the inner vertical bristle than to the proclinate orbital bristle; (2) third costal section with thornlike warts; (3) middle tibia with longitudinal rows of minute cuneiform bristles; (4) ninth abdominal tergite of female bilobed; (5) egg-guide lobes fused with each other completely or to great extent; (6) egg guide without developed teeth but with hairs (among Drosophilinae, Microdrosophila and Mycodrosophila are exceptional in comprising species that have no teeth); (7) male clasper without teeth (among Drosophilinae, Drosophila (Sophophora) alpina Burla is exceptional in having no teeth).

Oshima, C. Analysis of DDT resistance in D. melanogaster by population genetics.

DDT resistance in D. melanogaster is thought by workers to be a complex polygenic character controlled by genes on two large autosomes. The original strains used in this experiment had DDT resistance (survival rates in DDT test) as follows: Hikone, 98.0%; Kanmurijima, 72.0%; Canton-S, 0.0%; sca:ss<sup>a</sup>, 32.0%. The DDT resistance of these strains was analyzed by the use of a mutant tester, sca:ss<sup>a</sup>. Two kinds of populations were produced. One had heterogeneous second chromosomes of wild and tester strains and homogeneous third chromosomes having the ss<sup>a</sup> gene. The other had homogeneous second chromosomes having the sca gene and heterogeneous third chromosomes of wild and tester strains. DDT-resistance in these analytic populations was tested after 120 and 360 days without exposure to DDT. Although the frequencies of the mutant genes were gradually decreased by natural selection, the DDT resistance of the initial populations was retained. After maintenance for 160 days, the analytic populations were combined in pairs, and the DDT resistance of the combined populations was tested. The results are shown in the table.

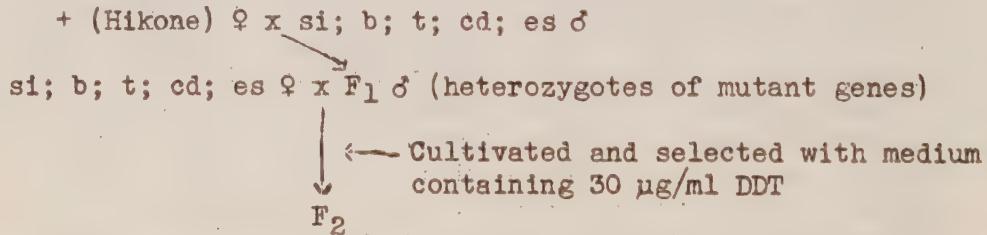
(See p. 152 for table)

Analytic population		Survival	Combined	Survival
2nd chr.	3rd chr.	rate (%)	population	rate (%)
sca	ss <sup>a</sup>	A: 27.9	A + B	: 45.4
Canton-S	ss <sup>a</sup>	G: 43.1		
sca	ss <sup>a</sup>	B: 31.1	G + H	: 68.1
sca	Canton-S	H: 48.9		
sca	ss <sup>a</sup>	C: 60.5	C + D	: 85.8
Kanmuri.	ss <sup>a</sup>	I: 66.5		
sca	ss <sup>a</sup>	D: 46.3	I + J	: 98.4
sca	Kanmuri.	J: 63.3		
sca	ss <sup>a</sup>	E: 86.1	E + F	: 97.6
Hikone	ss <sup>a</sup>			
sca	ss <sup>a</sup>	F: 70.5	E + L	: 92.3
sca	Hikone	L: 61.9		

From the results it appears that the DDT resistance associated with genes on the 2nd chromosome was higher, though more variable between strains, than that associated with third-chromosome genes. The combined populations show heterosis with respect to DDT resistance.

Oshima, C., and Hiroyoshi, T.  
Gene analysis of DDT resistance  
in D. virilis.

localization of genes controlling this character by matings with a multi-chromosomal mutant strain, as follows:



All F<sub>2</sub> flies that emerged from DDT-free medium and the DDT medium were divided into sixteen kinds of mutant flies according to phenotype, and counted. The results are shown in the table.

(See p. 153 for table.)

It may be concluded that dominant genes for DDT resistance are located in the second and fifth chromosomes, and that their effects are almost the same.

Phenotype				Control (culture medium contained no DDT)			Selection with DDT medium (30 µg/ml)		
Chromosome				♀	♂	Total	♀	♂	Total
2	3	4	5						
+	+	+	+	115	114	229	54	24	78
b	+	+	+	104	90	194	19	13	32
+	t	+	+	67	95	162	31	36	67
b	t	+	+	63	70	133	12	8	20.
+	+	cd	+	96	87	183	34	32	66
b	+	cd	+	57	53	110	9	9	18
+	t	cd	+	81	90	171	40	38	78
b	t	cd	+	70	63	133	11	6	17
+	+	+	es	88	83	171	8	6	14
b	+	+	es	53	66	119	3	0	3
+	t	+	es	74	86	160	9	13	22
b	t	+	es	57	55	112	4	4	8
+	+	cd	es	64	71	135	24	5	29
b	+	cd	es	42	39	81	4	2	6
+	t	cd	es	80	67	147	7	19	26
b	t	cd	es	61	56	117	10	0	10
Total		1172	1185	2357			279	215	494
Percentage of									
total	b			42.4%					23.1%
total	t			48.2%					50.2%
total	cd			45.7%					50.6%
total	es			44.2%					23.9%

Oster, I. I. A technique for treating male and female germ cells with chemical mutagens.

Because of the variations in sensitivity to mutagens of the different stages of male (Auerbach, 1954) and female gametogenesis, it is desirable to treat samples of germ cells which are as nearly homogeneous as possible. One way in which this can be done is by injecting the chemical to be tested into the hemocoel of inseminated females, inasmuch as the work of Abrahamson and Telfer (MS in preparation) has indicated that spermatozoa stored in the female are relatively uniform developmentally; and--so far as the female germ cells are concerned--it has been found that females fed on a protein-deficient medium (sugar-agar food) for about 10 days will have mostly oögonial stages in their ovaries at the time of treatment. On the other hand, if one wishes to treat more mature germ cells of the females, they should be kept on richly yeasted food and the offspring arising from eggs laid 1-2 days after treatment can be tested for lethal mutations. In the latter case, however, insufficient time may have elapsed for some chemicals to diffuse to and act on the sperm stored in the spermathecae and seminal receptacles. Thus the most suitable procedure for each chemical should be determined empirically.

Using this method we have injected triazine (2:4:6-tri(ethyleneimino)-1:3:5--triazine) into 10-day-old "multi" females (Muller, DIS-28) that had been inseminated by "multi" males from the 10th to the 13th day after hatching.

During their whole imaginal life until treatment, and until 5 days after treatment, these females were kept on sugar-agar food. The following results were obtained by examination and testing of the  $F_1$  derived from them:

	% X- or Y- chromosome losses (paternal origin)	% lethal mutations		% translocations (paternal origin)
		(paternal origin)	(maternal origin)	
triazine (0.06%) (in 0.4% saline)	$\frac{4}{797} = 0.50$	$\frac{30}{657} = 4.57$	$\frac{8}{657} = 1.22$	$\frac{8}{626} = 1.28$
control (0.4% saline)	$\frac{1}{1712} = 0.06$	$\frac{8}{1415} = 0.57$	$\frac{6}{1415} = 0.41$	$\frac{0}{1195} = 0$

These results indicate that triazine is mutagenic to sperm stored in the spermathecae of females, and suggest that it is weakly mutagenic to oogenesis. As the results from the tests of the female germ cells do not yet appear to be decisive, additional work using both immature and mature female germ cells is currently under way.

It is realized that with some agents this method will not be feasible because the chemical within the hemocoel may be too toxic or because it may be destroyed before reaching the sperm or may not even be able to pass through the walls of the spermathecae, seminal receptacles, and/or oviducts. The barriers to passage of the chemical to the sperm in the spermathecae may however be less for some chemicals with this method than with other methods. Moreover, the present method allows for the treatment of homogeneous samples of sperm while at the same time giving a relatively good opportunity for the production of effects on the female germ cells.

(This work has been supported by a grant received for work of Dr. H. J. Muller and associates from the American Cancer Society, on recommendation of the Committee on Growth of the National Research Council.)

Oster, I. I. Breeding scheme for studies of mutagenesis in males and females.

A scheme incorporating Muller's "multi-purpose-type" stocks (DIS-28) for studies on the production of lethals, translocations, and losses of chromosomes and chromosome parts, which also allows for an estimation of the frequency of lethals induced in a rod as compared with a ring-X chromosome in the same individuals, may prove useful in experiments similar to those under way in our laboratory on the analysis of the processes occurring after different types of combination treatments of *Drosophila* germ cells.

For this purpose three new stocks have been constructed, designated "Multipare," "Mutipare D," and "Mutipare R" (to denote the words multi-purpose stocks for comparing the frequencies of induced mutations in rod and ring X chromosomes), which can be used as follows:

$P_0$  y sc In49 B<sup>M1</sup>; twl bw; st<sup>54i</sup> ("Multipare" Bloomington \$ j99) virgin ♀ x sc<sup>8</sup>.Y/X<sup>c2</sup>, y; twl bw; st<sup>54i</sup> ("Multipare R" Bloomington \$ j100) ♂  
 $P_1$  X<sup>c2</sup>, y/y sc In49 B<sup>M1</sup>; twl bw; st<sup>54i</sup> virgin ♀ x sc<sup>8</sup>.Y/y sc<sup>S1</sup> B InS ("Multipare D" Bloomington \$ f46) ♂

↓  
 fertilized females (can be treated)

The expected "regular" classes of  $F_1$  flies are: x<sup>c2</sup>, y/sc<sup>8</sup>.Y ♂ (y<sup>+</sup>)  
 y sc In49 B<sup>M1</sup>/sc<sup>8</sup>.Y ♂ (y<sup>+</sup> sc B<sup>M1</sup>)  
 x<sup>c2</sup>, y/y sc<sup>S1</sup> B InS ♀ (y B)  
 y sc In49 B<sup>M1</sup>/y sc<sup>S1</sup> B InS ♀ (y sc B)

The frequency of yellow males will indicate the rate of loss or partial loss of the sc<sup>8</sup>.Y (which contains the normal allele of y), and the appearance of y sc<sup>S1</sup> B InS males will be an indication of the frequency of the loss of either of the maternal chromosomes by breakage, lagging, or nondisjunction. The frequency of nondisjunction in the  $P_1$  female is separately disclosed by the non-yellow  $F_1$  females produced.

For the detection of sex-linked lethals, the  $F_1$  females should be separated into two groups according to the marker, scute (sc), thereby distinguishing the lethal tests of the maternal rod-X from the maternal ring-X-chromosome. They may be mated individually as nonvirgins to the sc<sup>8</sup>.Y/X<sup>c2</sup>, y and sc<sup>8</sup>.Y/y sc In49 B<sup>M1</sup> males respectively. The absence of y<sup>+</sup> and/or y<sup>+</sup> B males will indicate the presence of a lethal in the chromosome of maternal origin and/or paternal origin, respectively. The presence of twl, bw, and st<sup>54i</sup> in the male stocks will not hamper the classification of y<sup>+</sup> and B; any nondisjunctionally produced males can easily be disregarded, as such flies, lacking the sc<sup>8</sup>.Y, will be yellow.

In order to detect any induced translocations the two types of  $F_1$  males can be mated individually to two or three virgin y sc In49 B<sup>M1</sup>; twl bw; st<sup>54i</sup> females of the "Multipare" stock. The absence of one or both classes of recombinants involving any of the three pairs of markers (y vs. y<sup>+</sup>, twl vs. twl<sup>+</sup>, and st vs. st<sup>+</sup>) considered two pairs at a time will then indicate the presence of a translocation between the chromosomes with those markers or their alleles. For the detection of translocations of the Y chromosome with either of the two large autosomes, it is preferable to use y vs. y<sup>+</sup> as a marker pair in addition to sex because of the frequency with which translocated Y's undergo nondisjunction with X's. As a matter of fact, this tendency should allow many of the Y-IV translocations to be picked up, when both sex and body color are taken into consideration, even though the IV itself lacks a marker. Matings yielding insufficient flies for the determination of whether or not a lethal or translocation is present should be retested by repeating with the  $F_2$  flies crosses like those of the  $F_1$ .

This set-up offers the special advantage of having the easily discernible markers, yellow body color (y), Bar eyes (B), twirled wings (twl) (Meyer, DIS-29), and scarlet eyes (st<sup>54i</sup>) which will appear white in combination with brown (bw); and thus a routine observer can easily do the scoring with a hand lens (magnification 4x), if not with the naked eye.

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Oster, I. I. "Tam"--a translocation-bearing stock for accumulating mutations in the male.

As early as 1918 Muller (1928, *Genetics* 13: 279-357) had devised and utilized breeding techniques for the accumulation of recessive mutations in the autosomes of *D. melanogaster* which had the advantage

of being automatic (i.e., requiring no selection by the experimenter while the mutations were being accumulated over succeeding generations), but these schemes did not allow one to distinguish the mutants arising in the male from those occurring in the female. More recently he has described stocks called "plond" (DIS-22), "plynd" (DIS-23), "jynd" (DIS-24), and "facl" (DIS-28), which are very useful in studies involving the accretion of lethals and visibles arising in the sex chromosomes of females, and in this issue stocks for the detection of mutations in the third chromosome.

In dealing with low mutation frequencies it is not only advisable to use techniques which allow for the accumulation of mutants over a period of generations but it facilitates matters if large segments of the chromosome complement can be analyzed for such mutations. Thus a stock enabling us to test one of the autosomes would yield about twice as high a mutation rate as one using only the sex chromosome, each autosome being approximately twice the size of the X chromosome. If the mutations are transmitted through the male line there would be no danger of losing any of the lethals via crossing over. Furthermore, as the stages of gametogenesis and other conditions under which mutations occur may vary from one sex to the other, it would be desirable to have stocks for accumulating mutations in the male.

Although it has been shown that a large majority of translocation homozygotes are inviable or sterile because of position effects on genes near the breaks of the rearrangement, it is possible to obtain stocks which do not differ from these ill effects if enough individual cases are examined. By irradiating a stock containing the recessive mutation, red (DIS-28, red malpighian tubules), we obtained a translocation involving the X and the third chromosome which is fully viable and fertile in homozygous females whereas aneuploids for this rearrangement do not survive. Although we used the marker, red, because it serves to distinguish first-instar larvae homozygous for it and thus might be useful in developmental studies of the lethals obtained with this scheme, other easily distinguishable mutations can be used instead. In crosses of males with this translocation to  $y\ f\ f$  females it is transmitted to the male offspring exclusively in every generation. In order to avoid, in the test crosses of the next-to-last generation, the obtaining of fertile females containing inversions in two chromosomes--a situation which would tend to increase crossing over and the danger of losing some of the lethals--singed (sn), which causes females homozygous for it to be sterile, was crossed into the X of the "Tam" males.

"Tam X3sn" (Bloomington \$ j17 in DIS-29) treated or untreated males are crossed to  $y\ f\ f$  females for one or n generations, depending on the nature of the experiment, and each generation of flies can be merely shaken over as no selection is required. After this period, the following crosses are carried out in order to test for mutations:

(See p. 157 for crosses.)

sn red D<sup>3</sup> Sb  
 P<sub>1</sub> ----- (") dd x y f: --- \*  
 ( ) -----  
 InLP Dfd InRP ca

sn red y sc<sup>S1</sup> B In49 1 sn<sup>x2</sup> sc<sup>8</sup> ru h D InsCXF \*\*  
 F<sub>1</sub> ----- (") d x 2 or 3 -----  
 D<sup>3</sup> Sb 1<sup>J1</sup> sc<sup>J1</sup> oc ptg B<sup>M1</sup> Me, Ins ri Sb<sup>1</sup>  
 (D Sb) Y+

\* ("Tam tester 1") ♀ (Bloomington ♀ j14)  
 \*\* ("Tam tester 2") ♀ (Bloomington ♀ j15)

sn red sn red  
 F<sub>2</sub> ----- (") d x -----  
 D, Ins or Me, Ins 1<sup>J1</sup> sc<sup>J1</sup> oc ptg B<sup>M1</sup> D, Ins or Me, Ins

(shake over)

F<sub>3</sub> look for and/or at red ♀ for lethals and/or visibles, respectively.

In the F<sub>1</sub> cross it is necessary to pick out the D<sup>3</sup> Sb males which are crossed individually to "Tam tester 2," which contains inversions in both X chromosomes, in order to counteract the increase in crossing over caused by the presence of inversions in the third chromosomes. Because aneuploids for the translocation are inviable and because the "Tam tester 2" females, being heterozygous for inversions of the X and containing an extra Y chromosome, undergo a high frequency of nondisjunction, considerable numbers of sons bearing the father's X (and necessarily the third chromosome to which it is translocated) are obtained, as well as normally produced females also heterozygous for the translocation. The unwanted types (D<sup>3</sup> Sb/ru h D InsCXF or D<sup>3</sup> Sb/Me, Ins ri Sb<sup>1</sup>) do not survive because the combinations D<sup>3</sup>/D and Sb/Sb<sup>1</sup> are lethal and the series of inversions in the D or Me chromosome effectively prevents crossing over with the translocated third chromosome. As mentioned before, the presence of sn prevents the females with inversions in heterologous chromosomes from breeding, thereby eliminating any chance for increased crossing over. Thus it is only necessary to transfer the F<sub>2</sub> generation to new vials and to look at the F<sub>3</sub> for the absence of red females, indicating the presence of a lethal, or to examine the red females for visible mutations.

Periodically it is advisable to free the original "Tam" stock lacking sn of newly arisen mutations by making it homozygous for the translocation and selecting the most viable line or lines in order to avoid starting new experiments with any pre-existing lethals. In practice this is most readily done by crossing to females with attached X's (y f:=) the males obtained in F<sub>3</sub> from those test crosses that gave the most nearly normal results.

Stocks containing a translocation of the X, second, and third chromosomes can be constructed which can be used for an analysis of both autosomes, thereby adding considerably to the significance of the relatively low spontaneous mutation rates which one may be dealing with. Such stocks should be useful in the study of the incidence of spontaneous mutations occurring under different conditions and for the experimental induction of autosomal mutations, because as in the case with "Tam X3" described above no selection is needed during the generations when this necessity has usually limited the

amount of data obtainable from this type of experiment.

It should be borne in mind that the lethal frequencies obtained by means of such accumulator schemes should be corrected by a factor representing the amount of selection which has acted against the heterozygotes over the generations because of the partial dominance of the lethal recessives.

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Oster, I. I., and Cicak, A. Z.

The frequency of spontaneous changes in morphologically different sex chromosomes.

In order to determine whether the spontaneous rates of chromosome loss and lethal frequencies are in part dependent on chromosome morphology, three stocks, each containing a  $sc^8.Y$  but having a different type of sex chromosome, were made up. Their composition was as follows:

$sc^8.Y/v$

$sc^8.Y.X^{c2}, y/v$

$sc^8.Y/Y^S. Dp R y X^+ bb. Y^L$  (Lindsley and Novitski, 1950, DIS-24 et seq.).

Males 0-12 hours old from a line selected for equivalent developmental rates and productive capacities from each stock (in order to rule out biasing the results by the formation of "synthetic" lethals in those stocks which might contain pre-existing detrimentals) were mated to 6-day-old  $y^{Si} sc^8 B f In49 v; bw$ ;  $e$  virgin females, one male to two females per vial for 3 days. The  $F_1$  males were examined from each vial separately in order to verify the presence of  $sc^8.Y$  in the  $P_1$  males. The lethal tests of the  $F_1$  yellow ( $y$ ) Bar ( $B$ ) females from each  $P_1$  male cross were kept together, thereby allowing for the recognition of "bunches" (i.e., cases representing a single mutation that had occurred at a very early embryonic stage of a parental male) of exceptional males or lethals. The lethal tests were made by crossing the  $F_1$  nonvirgin females individually to the  $F_1$  males.

As the  $sc^8.Y$  contains the normal allele of yellow ( $y^+$ ), all the  $F_1$  males should be gray ( $y^+$ ), Bar ( $B$ ), forked ( $f$ ), and vermillion ( $v$ ); any  $F_1$  males which were yellow ( $y$ )  $B f v$  represented cases of loss of the paternal  $X$  or  $Y$ . The absence of  $y^+$  males (normal with regard to  $B$  and  $f$ ) from the  $F_2$  cultures indicated the presence of a lethal in the chromosome derived from the  $P_1$  male. The frequencies of  $y B f v$  males in the  $F_1$  from matings of males containing a rod, ring, or "heterod" (extra-heterochromatin-bearing  $X$ ) were  $0.10 \pm 0.05\%$  ( $4/3948$ ),  $0.87 \pm 0.14\%$  ( $35/3984$ ) and  $0.03 \pm 0.03\%$  ( $1/2734$ ), respectively, whereas the lethal frequencies were  $0.29 \pm 0.09\%$  ( $12/4082$ ),  $0.30 \pm 0.10\%$  ( $10/3313$ ), and  $0.13 \pm 0.09\%$  ( $2/1538$ ), respectively. As all three stocks had a  $sc^8.Y$  any differences in their offspring would have been a reflection of the spontaneous behavior of their  $X$  chromosomes. In agreement with what had been found earlier, the ring- $X$  was lost the most frequently, presumably by twisted restitution or lagging, but the offspring of the rod and  $Y$ -bearing  $X$  stock showed no significant difference in their rates of yellow exceptional males. Several workers have shown that after radiation has been applied the heterochromatic areas undergo a disproportionately large number of rearrangements in relation to their size; and, although most of their evidence tends to support the view that this is due to a greater incidence of recombinational unions by this type of chromatin, it is possible that a greater susceptibility to breakage plays a role here. Although our results are not conclusive on this point, they do indicate, at least, that spontaneous breakage in the heterochromatin of the  $Y$  chromosome either occurs infrequently or at least in infrequently accompanied by euchromatic breaks whose ends undergo union with those derived

from the heterochromatic breaks. The fact that the three types of sex chromosome which because of their structure would be expected to behave differently when broken had similar lethal frequencies is an indication that breakage is relatively unimportant in the formation of spontaneous recessive lethal mutations.

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Oster, I. I., and Iyengar,  
Shanta V. A marked ring-Y.

After the irradiation of males containing "Cooper's Y" ( $sc^8(y^+).Y^S.Y^L.bw^+$ ) with 4000 r according to a scheme proposed by Muller, and the examination of about 2500  $F_1$  males, 121 individuals were found which lacked either  $y^+$ ,  $bw^+$ ,  $ss^+$  (the normal alleles, as a group, of the genes of  $Y^S$  whose absence results in sterility),  $sL^+$  (the corresponding ones of  $Y^L$ ), or a combination of these. While the genetic analyses were under way Miss Iyengar undertook a cytological examination of the chromosomes in the ganglia of several of the exceptional males and found that one of them, lacking  $y^+$  but retaining  $bw^+$ , was a ring. This Y, designated  $Y^c:bw^+$  or more specifically by the "signal" "MYR" (to signify the words a marked Y in the form of a ring), contains all the fertility genes of  $Y^S$  and  $Y^L$ . It was probably formed by loss of the  $sc^8$  segment from the  $Y^S$  and the tip beyond the fertility genes from the  $Y^L$  and subsequent reunion of the broken ends of the two arms of the Y.

Our intention in obtaining a marked  $Y^c$  chromosome was that it should prove useful in determining whether rod and ring Y's exhibit differential responses under the influence of various conditions.

(This work was supported by a grant to Dr. H. J. Muller and associates from the Atomic Energy Commission, Contract AT (11-1)-195.)

Plaine, Henry L. A sex difference in the frequency and expression of erupt eyes in the Swedish-b strain of *D. melanogaster*.

Practically all laboratory and wild strains of *D. melanogaster* tested carry the mutant gene "erupt" on the third chromosome and the suppressor of erupt on the second. The erupt mutant becomes manifested when the action of its suppressor is blocked by certain treatments (J. Genet. 53: 244, 1955). Regardless of the strain tested, or the treatment used, no differences between males and females have been observed with respect to the erupt eye condition.

Irradiation of ten-hour-old embryos of the Swedish-b strain with 1000 r units of X-rays produced an incidence of 89 per cent erupt eyes in the resulting adults. No other irradiated wild strain gives as high a frequency of affected individuals. This, together with results from genetic tests against the suppressor-erupt strain (Su-er bw; st er) (Glass, unpub.), indicates that the Swedish-b strain carries a strong allele at the erupt locus but a rather weak, though effective, allele at the suppressor locus. When three females with erupt eyes were discovered in this strain, they were isolated and a rigorous program of selection for the erupt phenotype was started in order to establish an erupt strain having an otherwise wild-type background (Su-er<sup>+</sup>; er). The present erupt strain comprises the third chromosome from bw; st and the second chromosome from al b c sp<sup>2</sup>, which is one of the few strains lacking the erupt mutant and its specific suppressor.

It soon became apparent, however, that loss or mutation of the second-chromosome suppressor could not alone account for the manifestation of the mutant. No erupt males were obtained until the third generation, after which they have been recovered repeatedly in seven successive generations, but the expression is always considerably more extreme in the females. Moreover, there is a significant difference between the frequencies of affected males and females in every case. In five different series, during the last three generations of each, the frequencies of erupt eyes ranged from 61.1% to 70.8%--with 23.5% to 26.7% of the affected flies males, and 36.7% to 46.7% females. It thus appears that the maximum frequency of erupt has been attained in this strain, at least by selection.

In each case the development of the culture follows a specific pattern. Of the flies eclosing on the first and second days, at least 50% of the females are erupt whereas less than 35% of the males are affected. On successive days the frequency of erupt flies of both sexes increases until finally 79% to 88.9% of the females and 44.4% to 57.4% of the males show the erupt phenotype. Thus, about three-fourths of the females develop erupt eyes whereas only about one-half of the males express the mutant. The over-all sex ratio is normal. Any hypothesis at this time would be purely speculative, but it appears that modifiers other than the suppressor gene are concerned in the present case.

Rasmuson, M. *Drosophila* species found at Uppsala, Sweden.

In a preliminary inventory of *Drosophila* species, traps containing fermenting bananas or standard *Drosophila* food were set up in the garden of the Genetical Institute at the Royal Agricultural College near Uppsala. The garden measures about 24 acres and contains fruit trees as well as many different kinds of foliiferous trees and shrubs. It is surrounded by open fields and woodlands. The trapping was started at the end of June and continued until the middle of September. During the whole period the weather was unusually hot and dry, with less than 10 mm total rainfall.

Ten different species were identified; their relative numbers are shown in the table. The species *D. obscura* and *D. subobscura* are recorded together because they were not classified separately in the earlier captures. When distinguished, *D. obscura* was always the most frequent. The species *D. nitens* and *D. guyenoti* are new for Sweden.

Some seasonal fluctuations were revealed. The species *D. obscura-subobscura*, *D. buskii*, and *D. phalerata* became less frequent as the summer proceeded, whereas *D. melanogaster* and *D. funebris* were most numerous in September. It may be that these fluctuations depended not so much on the seasonal change as on the increasingly severe drought. The investigation will be taken up again next summer.

(See table, p. 161)

	Total number	Frequency (%) during		
		June-July	August	September
D. <u>obscura</u> - <u>subobscura</u>	233	63.9	54.3	23.2
D. <u>melanogaster</u>	142	3.0	26.5	64.0
D. <u>buskii</u>	36	21.8	3.2	0
D. <u>funebris</u>	29	0	7.8	9.6
D. <u>nitens</u>	16	4.5	3.7	1.6
D. <u>phalerata</u>	11	6.8	0.9	0
D. <u>guyenoti</u>	5	0	1.4	1.6
D. <u>tristis</u>	4	0	1.8	0
D. <u>alpina</u>	1	0	0.4	0
Total numbers	477	June-July	August	September
		133	219	125

Redfield, Helen. Like recombination for yellow-split in four homozygotes for the white locus.

Previous results (DIS-28, 1954) had shown a significant difference in yellow-split recombination in the combined presence of Curly and Payne inversions for  $y\ w^{51a}/w^{51a}$  spl females ( $6.6 \pm 0.42$ ) as compared with  $y/spl$  ( $11.1 \pm 0.51$ ) and  $y\ w^{48h}/w^{48h}$  spl ( $11.4 \pm 0.43$ ) females. It seemed desirable to repeat these crosses. The new data give like  $y$ -spl recombination values in four different homozygotes for the white locus; that is, for three separate homozygous whites, including  $w^{51a}$ , and for the homozygous wild type allele of white. Aside from the white locus the stocks used were presumably isogenic (as they were in the previous crosses), and the presence of the inversions was freshly tested (by Dr. Jack Schultz) by cytological examination of salivary-gland cells. The actual  $y$ -spl values were: for  $y/spl$ ,  $9.4 \pm 0.66$ ; for  $y\ w^{48h}/w^{48h}$  spl,  $10.2 \pm 0.78$ ; for  $y\ w^{51a}/w^{51a}$  spl,  $9.9 \pm 0.66$ ; and, involving an allele ( $w^{53a}$ ) not previously used, for  $y\ w^{53a}/w^{53a}$  spl,  $9.6 \pm 0.48$ . The low value for  $w^{51a}$  in the earlier experiment is of course explainable on the assumption that one (or more) of the inversions was absent in the  $y\ w^{51a}/w^{51a}$  spl females tested at that time, the inversions being properly present in the rest of the females; on the other hand, there is reason for believing that the inversions were properly present also in these  $w^{51a}$  homozygotes, and that the true explanation involves another problem. However, as the case now stands there is an apparent discrepancy in the two sets of data; the later results show no difference in yellow-split recombination in homozygotes for  $w^+$ ,  $w^{48h}$ ,  $w^{51a}$ , and  $w^{53a}$ .

Rendel, J. M. Effect of low temperatures on mutation rate.

Attempts have been made to freeze *Drosophila* eggs to  $-79^{\circ}$  in order to study the effect of extreme temperatures on the mutation rate. A very small percentage of the eggs has so far survived this treatment, and the numbers of offspring produced have been small. There is no indication of any marked effect on the mutation rate of the X chromosome of D. melanogaster. Treatment of adult males at temperatures down to and including  $-30^{\circ}$  has so far given no indication of an increase in sex-linked lethal mutations.

When crossing-over frequency is increased in the X chromosome by the inclusion of the Cy inversion, the slight increase in coincidence is less than

is to be expected from the increase in coincidence found when the crossover frequency is increased by the inclusion in the marked area of a longer piece of chromosome.

Sandler, L. An attached-XY chromosome which shows no meiotic loss in the absence of a homolog.

It has been demonstrated previously (Sandler and Braver, Genetics, 1954) that certain of the attached-XY (YSX.YL) chromosomes are lost in the absence of a homologue. A new YSX.YL chromosome has

recently been synthesized, and, in the test to be described below, gives no good evidence of such loss. The attached-XY chromosome, in this case, was produced by an exchange between a chromosome in normal sequence carrying YL proximally, and one carrying the FR1 tip. The FR1 tip has been shown by Braver to be YS, but the exact mode of its origin is not yet known. The YSX.YL chromosome so produced has been designated FR1.YL and is marked by  $y\ cv\ v\ f$ . The chromosome has been tested in the following way: FR1.YL,  $y\ cv\ v\ f$  males, both with and without  $sc^8.Y$  (Muller, DIS-22) were crossed to homozygous  $y$  females. The progeny from males not carrying  $sc^8.Y$  included: 946  $y\ ♀$  and 1,048  $y\ ♂$ . The progeny from males carrying  $sc^8.Y$  included: 1,581  $y\ ♀$ , 1,796 +  $♂$ , 7 +  $♀$ , and 92  $y\ ♂$ . The difference between these two experiments is not significant. It seems most reasonable to conclude, therefore, that the FR1.YL chromosome in the absence of a homologue is not lost to any appreciable extent.

Sandler, L. Attempts which have failed to produce compound ring-X chromosomes.

During the course of the past year, two large experiments were performed for the purpose of synthesizing the two possible types of compound ring-X chromosomes

(i.e., the reversed ring and the tandem ring). Both these experiments failed. They are reported here for the reason that they represent the most obvious ways to attempt to synthesize the desired compound, and the failure of these attempts suggests that other, and presumably less obvious, methods will have to be employed.

The first experiment involved the following steps. Females heterozygous for (a) a chromosome in normal sequence marked with  $y\ w^a\ m$  and carrying the FR1 tip (=  $Y^S$ ; Braver), with the right end of  $T(1,4)\ B^S$  as a second arm, and (b) a chromosome carrying the  $sc^8$  inversion marked by  $cv\ v\ f\ car$ , with the long arm of the Y chromosome attached to the centromere (=  $FR1\ y\ w^a\ m.B^S/sc^8\ car\ f\ v\ cv.Y^L$ ), were either given approximately 2400  $r$  of X-irradiation or left untreated. Both types of females were crossed individually to  $y\ w^a\ cv\ m\ f\ car$  males. The progeny from these crosses were examined for  $B^S$  females and for + females.

If a heterochromatic exchange occurred between the FR1 tip and the base of the  $sc^8$  chromosome, when they were paired in such a way as to give recoverable products (Sandler, Genetics, 1954), there would be produced a reversed acrocentric compound X chromosome (= double-X) heterozygous for  $y\ w^a\ cv\ v\ m\ f\ car$ , which would carry the distal  $sc^8$  heterochromatin at the free end and the  $B^S$  duplication appended to the centromere. Such females would be phenotypically  $B^S$ . If such compounds were produced, then a single exchange between the  $B^S$  duplication and distal  $sc^8$  tip could result in a reversed compound ring-X chromosome which would be phenotypically recognizable because of the simultaneous loss of  $B^S$  and  $y^+$ .

If an exchange (in the original type of female) occurred between the base of the  $sc^8$  chromosome and the  $B^S$  duplication of the other chromosome, or an exchange occurred between  $YL$  on the  $sc^8$  chromosome and the base of the chromosome in normal sequence, there would be produced a tandem metacentric compound X chromosome (= tandem attached-X) heterozygous for  $y\ w^a\ cv\ v\ m\ f\ car$ , carrying the  $FR1$  tip on one arm and the  $sc^8$  heterochromatin at the other tip. Such females would be phenotypically wild type. In such a compound, an exchange between the two tips (paired in reverse) could result in a tandem compound ring-X chromosome, which would be phenotypically recognizable because of the loss of  $y^+$ .

Approximately 32,000 female progeny from unirradiated parental females and approximately 10,000 female progeny from irradiated parental females were examined. No reversed acrocentric compounds were recovered from either set. No tandem metacentric compounds were recovered from the unirradiated females, but four such compounds were recovered from the irradiation run. Stocks were made of each of these lines, and females from these stocks were X-rayed (ca. 2400 r) and crossed to  $YSX.YL, y\ B$  males. The progeny from this cross were examined for possible tandem rings (that is,  $y\ \text{♀}$ ). Seven  $y$  females which proved to carry unstable compounds (i.e., they regularly generated single chromosomes) were recovered from the irradiated females. Cytological examination of larval neuroblast cells of each of these recovered compounds showed that the compound possessed free ends and was therefore not a ring. There are a number of possible ways for these  $y$  compounds to have arisen; but the matter was not pursued further.

The second attempt to synthesize the compound rings was essentially the same as that reported above, with the differences that In (1)  $sc^1$  was used in place of In (1)  $sc^8$ , and  $YL$  was appended to the centromere of the chromosome carrying  $FR1$ , the  $B^S$  duplication being attached to the other centromere. That is, the original female used was of the constitution  $sc^1\ car\ m\ y.B^S/FR1\ y\ w^a\ cv\ v\ f.YL$ . This experiment involved about the same numbers of progeny as the previous one. The results were essentially the same.

Scossiroli, R. E. The effects of the relaxation of selection on plateaued populations.

selection applied to two treated lines was successful whereas selection applied to two control lines, which received no X-ray treatment, was virtually ineffective. From about 27 sternopleural hairs at the beginning of selection, the level rose to about 43 hairs in one of the irradiated lines and about 33 hairs in the other. Fertility was very low in these lines. The control line that was still present at the end of the experiment (the other having been lost) had 27.8 as the average number of sternopleural hairs. These levels were maintained in spite of continued artificial selection for sternopleural hairs.

At this point it was important to see what happens to selected lines which have reached and are maintained at such homeostatic plateaus when artificial selection is interrupted and natural selection is allowed to operate. Artificial selection and irradiation were interrupted, and the two X-ray-treated lines and the control were kept in mass cultures, where natural selection could operate through survival in competition. Periodic tests for number of sternopleural hairs and for fertility were performed. The mean

In DIS-26 and -27 preliminary reports were given of selection experiments for sternopleural hairs in plateaued populations subjected to X-irradiation. In brief, X-ray treatment was effective, since

bristle number in the two lines previously irradiated showed a steady decrease of about 3 hairs, but very soon reached a plateau which they maintained for 20 generations, at a level higher than that presented by the same lines at the beginning of artificial selection under radiation--namely, 40 and 30 hairs, respectively, compared with 26-27 hairs. In the meantime, fertility rose steadily and reached different levels of stabilization in the two lines. The control line, which before the interruption of selection did not show any response, after the interruption of selection displayed a very limited decrease in number of sternopleural hairs and maintained with regard to fertility the same peculiar type of high variability previously exhibited.

The results of these experiments support the conclusion that new levels gained through response to selection after a forced advance in a plateau are not lost when selection is interrupted. After a short period of regression the population reaches a condition of stabilization at a level higher than that displayed when selection was started.

Shiomi, T. The time of action of ultrasonic-induced lethals in D. melanogaster.

The time of action of 20 ultrasonic-induced sex-linked recessive lethals balanced by Muller-5 have been determined, as shown in the following table.

There is no qualitative difference in effect between these lethals and other lethals of various origins.

Lethal stage	Strain	Dosage
Egg	<u>l(1)us-20</u>	560 Kc, 1500 v, 2.5 A, 5 min.
	<u>l(1)us-48</u>	450 Kc, 1500 v, 2.0 A, 5 min.
Larval	<u>l(1)us-2, 5, 6, 7, 11,</u> <u>12, 13, 14, 25, 26</u>	560 Kc, 1500 v, 2.5 A, 5 min.
Larval and pupal	<u>l(1)us-9, 15, 16, 17, 22</u>	560 Kc, 1500 v, 2.5 A, 5 min.
Larval, pupal, and imaginal	<u>l(1)us-46, 47</u>	450 Kc, 1500 v, 2.0 A, 5 min.
Any time during development	<u>l(1)us-33</u>	450 Kc, 1500 v, 2.0 A, 30 min.

Sire, Marjorie W.

Variation of chromosomes in Michigan and Nebraska strains of D. algonquin.

Standard, A-1, and B-3--appear to be the same as those reported by Miller (1939). (Standard and B-3 differ by two overlapping inversions, one of which includes the spindle attachment.) Two new small inversions were found in the Michigan material, one in the long arm of the C chromosome, and the other in the short arm of the C chromosome. Standard was the only sequence found in the Nebraska material.

A study of the salivary-gland chromosomes was made in 18 Michigan and two south-eastern Nebraska strains of D. algonquin. Five sequences were observed in the Michigan strains. Three of these--

Sobels, F. H. The effect of cyanide pretreatment on mustard-gas-induced mutation rates.

As mustard gas resembles X-rays in many of its effects, it seemed of interest to investigate whether cyanide has a similar enhancing influence on the mutagenic action of mustard gas. Oregon-K males were injected with sublethal concentrations of potassium cyanide, which by itself does not raise the spontaneous mutation rate (Sobels, loc. cit.). By differently marking the cyanide-pretreated and mustard gas-control males, simultaneous exposure of both groups of flies to the mustard gas was possible. Tests for sex-linked lethals were made by the Muller-5 method in three successive broods by mating the treated males to three fresh females at time intervals of three days. The results strongly suggest an enhancing effect of the pretreatment. In three out of four experiments the over-all mutation rate, taken over three broods, was higher after the cyanide pretreatment than in the mustard gas controls. As a possible interpretation of these effects, an increase of the oxygen tension in the pretreated germ cells may be taken into account, although this is in contrast to results obtained by Auerbach and Moser (*Experientia* 7: 341, 1951) and Kihlmann (*Intern. Radiobiolog. Conf.*, Cambridge, 1955).

Spofford, Janice B. The reversibility of X-ray-induced mutations.

Although inconclusive in themselves, the following results are recorded as an addition to the growing literature of unsuccessful attempts to induce reverse mutations in loci already altered by X-rays. The initial mutations were induced in males of an  $e^s$  stock maintained by brother-sister pair matings for a minimum of 7 generations. After exposure to 2000 r delivered at 250 Kv, 15 Ma with a Maximar machine, X-rayed males were crossed to  $y$ ;  $e^s$  females from a stock maintained by backcrossing to the  $e^s$  stock. Amongst 7993 sons bearing irradiated X chromosomes appeared 1 white, 3 ruby, 2 facet, 1 garnet, 1 white-mottled, 1 lozenge, 1 brown-dominant, 1 yellow, 2 rudimentary, and 7 phenotypically less clear mutations. Mutant lines were maintained by backcrossing to  $y$ ;  $e^s$  females.

The white mutant and one of the ruby mutants were selected as probable point-mutations for further X-raying, because of normal crossover rates and normal appearance of the salivary chromosomes. These mutant males were irradiated and tested by the same procedure as above. Among 31,744 sons of X-rayed white males were 13 which differed phenotypically. Twelve of these were still white-eyed; the other resembled ruby. None of the mutants was fertile when mated with  $y$ ;  $e^s$  females. The "ruby mutant" came from a well-stoppered bottle, but its sterility and the presence of ruby stocks in the laboratory do not permit the elimination of contamination as a possible explanation. Among 46,653 sons of X-rayed ruby males were 48 phenotypically abnormal sons, including 22 with altered eye colors. Seven of the males with mutant eye colors were fertile, the mutations segregating from ruby. In this fertile group were three independent mutations to white (in addition to five occurrences of sterile white males). One unusually small male had wild-type eyes and a sooty body color, but had anomalous marginal veins and died soon after eclosion, suggesting that the wild-type eye color was not the consequence of a simple reverse mutation at the ruby locus.

Previous experiments showed that substances which inhibit cytochrome oxydase and catalase, like cyanide and azide, exert an enhancing effect on X-ray-induced mutation rates (*Z.I.A.V.* 86: 399, 1955).

Stumpf, Hildegard. Preferential directions and their changes during development in the wing of D. melanogaster.

Pupal wings of different ages prepared in Ringer's solution were swelled in diluted Ringer's solution or shrunk in concentrated  $\text{Na}_2\text{SO}_4$  solution. In this process, the length and breadth of the wings of some stages did not change (increase or decrease, respectively) to an equal extent. Wings 16-18 hours old swelled more in length than in breadth, but shrank more in breadth. Wings 22-23 hours old behaved in the opposite way; they swelled more in breadth and shrank more in length.

The differences in swelling and shrinking of wings at the stages mentioned point to the existence of some linear structural elements, which at different times may show different preferential directions. In the beginning (16-18 hours) the preferential direction probably lies in the transverse axis of the wing, later on (22-23 hours) in the longitudinal axis. The structural elements contract in concentrated  $\text{Na}_2\text{SO}_4$  solution; and in diluted Ringer's solution the entrance of water primarily enlarges the distances between the structural elements lying in the preferential direction.

It is supposed that these preferential directions within the protoplasm play a role in forming the shape of the wing. Processes of wing development, such as contraction, expansion, and cell division, may have more influence on the length or on the breadth of the wing, depending on the phase in which they take place. In fact, a connection could be established between the direction of mitotic spindles and the preferential direction indicated by swelling and shrinking. In 16-18-hour-old wings, transversally oriented mitotic spindles prevail, corresponding to the presumed preferential direction in wings of this age. At the age of 22 hours, the end of the mitotic period, longitudinally oriented spindles prevail. Thus there is a change in preferential direction of the spindles during pupal development, which parallels the observed changes in swelling and shrinking.

Sturtevant, A. H. Failure of w or cn bw to suppress the nondisjunctional effects of cand.

cand females (Lewis and Gencarella, Genetics 37: 600-601). It seems possible that this result may be due to the blocking of a reaction in the synthesis of eye pigments. Accordingly, cand females were made up that lacked eye pigment because they were also w, in one experiment, or cn bw in another experiment. In both cases, the result was the same as from ordinary cand females--a high frequency of nondisjunction and of mosaics. This negative result is reported here to save other workers the trouble of repeating these particular tests.

Taira, T., and Nawa, S.  
The red eye pigment of D. melanogaster and pteridine derivatives.

In DIS-28, we reported on the close relation between the red or yellow eye pigment and pteridine compounds. Recently, Forrest and Mitchell (1954) tentatively proposed the structure 2-amino-4-hydroxy-7-hydro-8-lactylpteridine-6-carboxylic acid for the yellow eye pigment. Double recessive mutants, v;cl, v;se, and bw;se, were used for a chemical study on the relation between yellow or red eye pigment and pteridine derivatives. Mutant males 7 to 10 days after emergence were divided into heads and bodies, and homogenized with 30% acidic ethanol. The supernatant was analyzed

The mutant cand, like the ca of D. simulans, gives a high frequency of non-disjunction (both for X and for 4), and a high frequency of gynandromorphs and of haplo-4 mosaics, among the offspring of

by paper chromatography, using methyl ethyl keton, propionic acid, and water (75:25:30). The relative values of the yellow pigment and isoxanthopterin were measured by the fluorometric method, and the relative amounts of the red pigments I and II were estimated by extinction coefficient at 480  $\mu$  wave length. The results are shown in the following table.

	Head		Body isoxanthopterin
	Yellow pigment	Red pigment (I)*	
se	82	0	37
v;se	80	0	42
v	0	113	47
v;cl	42	26	41
cl	48	27	43
bw;se	0	0	0
bw	0	0	0

\* (I), higher Rf value; (II), lower Rf value.

From these results the following conclusions have been drawn: (1) v;cl is similar to cl in quantity of red or yellow eye pigment and in that of pterin; (2) v;se closely resembles se in quantities of yellow eye pigment and pterin; (3) neither bw;se nor bw has pteridine derivative.

The data suggest that the red eye pigment is a pteridine derivative, and that the yellow pigment is its precursor.

#### Taira, T. and Nawa, S.

Yellow pigment found in the body of the mutant "sed" in *D. melanogaster*.

The eyes of the mutant "sed" were analyzed chemically by means of chromatography and spectrophotometry. It was found that the pigment consists of both yellow and red eye pigments. On the other hand, the fluorescent substance contained in the body of the mutant is made up largely of yellow fluorescent substance and a small quantity of purple substance. The latter is nothing but isoxanthopterin. The occurrence of the yellow pigment in the body is apparently characteristic of this mutant, and not found in any other. The yellow pigment found in the body of "sed" has the chemical structure 2-amino-4-hydroxy-7-hydro-8-lactylpteridine-6-carboxylic acid, like the yellow eye pigment found in both se and cl. The male has this substance in its body in larger quantity than the female.

#### Tantawy, A. O. Crossing inbred lines maintained with different rates of inbreeding but at the same levels of homozygosity.

An experiment, consisting of crosses between random inbred lines of *D. melanogaster* (Crianlarich strain), was performed in order to determine the effect of such crosses on wing and thorax length and on percentage of emergence. Two systems of mating were carried out, namely, brother-sister and double-first-cousin matings. In each system, five parallel inbred lines were maintained, and, at the 25%, 50%, and 75% coefficients of inbreeding, were crossed within each system of mating. The ten possible crosses in each system were carried out. Throughout the experiment a control stock from the mass-mating population was maintained under the same environmental conditions as the inbred and crossed lines.

Before starting this experiment, heritability of wing and thorax lengths as well as the genetic correlation between them were determined, by two progeny tests. In the first progeny test mating was at random and in the other it was assortative, the results of the latter being corrected for the magnified variance between parents. These heritabilities are shown in table 1.

Table 1

Type of mating	Wing length	Thorax length
Random	37.22 ± 7.13	36.95 ± 7.37
Assortative	41.12 ± 10.20	39.95 ± 12.00
Weighted means	37.33 ± 2.00	32.00 ± 1.50

The weighted means of the heritability for both wing and thorax length indicate a high genetic variance, which is due to the additive gene effects. The genetic correlation between wing and thorax length, which describes the relation between the additive deviations caused by genes common to the two characters, was on the average 74.35%.

The inbred lines were crossed within each system of mating at the same or nearly the same coefficient of inbreeding, to study the amount of heterosis for body size and percentage of emergence. Heterosis was measured as percentage of deviation of the crossed lines from the unselected inbred ones. Analyses of the deviations of the inbred lines from the controls, with respect to wing length are presented in table 2.

Table 2

Coef. of inbreeding (%)	Brother-sister				Double-first-cousin			
	C.V. (%)		Deviation (%)	No. of flies	C.V. (%)		Deviation (%)	No. of flies
	Controls*	Inbred			Controls*	Inbred		
25	2.68	2.57	-0.70	200	2.26	2.40	-0.16	200
50	2.34	1.95	-2.77	191	2.06	1.92	-1.78	200
75	2.24	1.53	-3.06	195	2.39	1.92	+0.57	200

\* Forty pairs of flies.

It is clearly shown by the table that lines maintained with brother-sister matings suffered a greater inbreeding depression than those maintained by double-first-cousin matings. This was probably because the recessive genes become homozygous at a faster rate in the case of sib matings. A mutation which affects eye color appeared in one of the inbred lines of sib matings at 50% of inbreeding. This is a recessive, single-gene mutation, and it is under investigation. From this table it can also be seen that the coefficient of variation (C.V.) declined in both systems of mating from the control level; brother-sister matings showed a greater decline.

When the different inbred lines were crossed, heterosis occurred in both systems, but was greatest in the more rapidly inbred lines, as shown in table 3 with respect to wing length.

Table 3

Coef. of inbreeding (%)	Brother-sister				Double-first-cousin			
	C.V. (%)		Heter- osis (%)	No. of flies	C.V. (%)		Heter- osis (%)	No. of flies
	Parent	Offspring			Parent	Offspring		
25	2.64	2.00	+0.14	964	2.42	2.05	+0.25	1000
50	1.98	1.76	+1.12	1000	2.01	1.82	+0.41	1000
75	1.46	1.73	+2.72	908	2.02	1.70	+0.66	1000

Heterosis of wing length increased gradually with increased homozygosity, and brother-sister matings showed a higher percentage of heterosis at any level of homozygosity than double-first-cousin matings. Crossing did not generally result in body sizes significantly larger than those of the original non-inbred stock. In fact, wing length of the crossed lines never reached that of the controls. Under both systems of mating, coefficients of variation for the crossed lines were less than for the random lines.

Thorax length behaved in almost the same manner as wing length. In the case of percentage of emergence, heterosis was measured as the percentage of deviation of the crossed lines from the random inbred lines, as shown in table 4.

Table 4

Coef. of inbreeding (%)	Brother-sister		Double-first-cousin	
	% deviations from controls	Heterosis (%)	% deviations from controls	Heterosis (%)
25	-0.95	-2.98	-----	-----
50	-6.18	+7.12	-1.38	-0.05
75	-14.98	+11.19	-10.24	+5.91

Percentage of emergence in the case of brother-sister matings was the lowest at any level of inbreeding; and crosses between the inbred lines increased the percentage of emergence, especially at the higher levels.

The results indicate clearly that heterosis occurs when inbred lines are crossed, but that crossed lines hardly reach the level of the initial unselected stock. The appearance of heterosis depends on the depression effects of inbreeding on the character under investigation, and this depression may depend on the type of mating.

Heritability of wing and thorax length in the random inbred lines and the crossed lines will be analyzed to show the relation of genetic variance in these lines to coefficients of inbreeding.

Thoday, J. M. Homeostasis  
in a selection experiment.

From a wild stock of melanogaster six lines have been bred for 10 generations--two selected for high and two for low sternopleural number, and the other two used as controls. Each generation of each line was set up as four single-pair cultures, with rotational mating around the four cultures. Bilateral asymmetry of sternopleural number was

measured as difference between the sides divided by total bristle number. Asymmetry increased in the selection lines ( $p < 0.001$ ), with no difference between high and low lines. Asymmetry did not increase in the controls.

In most generations  $F_1$  hybrids were obtained between the pairs of lines and also between a high and a low line. Asymmetry increased with generation in the  $F_1$ 's between selected lines just as much as in the lines themselves. These results indicate that homeostatic mechanisms deteriorated in the selection lines but that this was not related to increasing homozygosity. It is concluded that the increase of asymmetry is caused by selection for low homeostasis, itself an inevitable result of powerful directional selection.

Toyofuku, Y., and Momma, E.  
A cloudy pattern on wings of  
D. nigromaculata.

This species is one of the most dominant *Drosophilids* in Hokkaido. The flies have dark brownish clouds on the tips of the third longitudinal veins. No such clouds

are found for a while after emergence. They generally make their appearance by 2 to 3 hours after emergence at  $23^{\circ} C$ , and the figures are settled between 20 and 24 hours. The clouds are characterized by various patterns. The frequencies of the different patterns observed in flies from the field showed variations that were either seasonal or geographical.

Ulrich, Hans. Comparative studies on the lethal action of X-rays on nucleus and cytoplasm of *Drosophila* eggs before cleavage.

Anterior and posterior halves of *Drosophila* eggs, 10-20 minutes old at the beginning of treatment, were X-rayed with various doses (50 Kv, 10 ma, 1 mm Cellon, focus distance constant, time of exposure varied from 32 to 480 seconds). At this

age the anterior half of the egg contains the nucleus (the two pronuclei) at stages varying between meiosis and first cleavage, whereas the posterior half does not yet contain any nucleus. The frequencies of nonhatching eggs were registered.

The two resulting dose-effect curves differed quantitatively and qualitatively (Biol. Zentr. 74: 498-515). The  $LD_{50}$  of the anterior half was about 182 times lower than the  $LD_{50}$  of the posterior half. (In earlier experiments with a more imperfect method it appeared to be 66 times lower; see DIS-28, pp. 164-165.) In order to compensate for this high difference in radiosensitivity, that is, to obtain comparable lethal frequencies with similar times of exposure, we irradiated the two halves at two different focus distances: 65 cm and 5 cm, respectively.

The dose-effect curve obtained by X-raying the anterior halves is nearly an exponential curve, that is, a straight line in semilogarithmic plot. In contrast the dose-effect curve obtained by X-raying the posterior halves rises concavely in semilogarithmic plot. After the 50% point, it rises very steeply, reaching the 99% point at a dose of about  $1.75 \times LD_{50}$ , whereas the anterior-half curve does not reach the 99% point before a dose of about  $6 \times LD_{50}$ .

It was thought that the specific shape of the posterior-half curve, that is, its steeper rise at high doses, might be due, exclusively or partially, to the fact that application of a higher dose required a longer time of exposure. During this longer time, development might have proceeded, so that cleavage nuclei might have migrated in some cases to the irradiated posterior half of the egg. Thus, this part of the egg might have grown more radiosensitive than

it was at lower doses (shorter times of exposure). In order to check this possibility, we irradiated halves of 5-10-minute eggs in the same manner as before--varying the dose by varying the time of exposure. Furthermore, we treated halves of 10-20-minute and 5-10-minute eggs in a constant time of 64 seconds, varying the dose by varying the focus distance (anterior half, 92 to 23.7 cm; posterior half, 7.1 to 2.24 cm). In all cases the posterior-half curve showed the same characteristic shape, which was different from the exponential curve of the anterior half. Hence, the qualitative difference of the two curves points to a difference in the mechanism of radiation action.

The exponential dose-effect curve resulting from X-raying the anterior half may be explained in terms of the target theory. Thus we may conclude that the egg is killed by a single-hit event. Probably this hit produces a dominant-lethal factor in the nucleus, namely, a chromosome break.

The concave curve resulting from irradiation of the posterior half can hardly be interpreted according to the target theory. The fact that its shape differs from that of the anterior-half curve indicates that the lethal action of X-rays applied to the posterior half is due to damage induced in the irradiated cytoplasm itself, rather than to scattered X-rays and secondary electrons that might reach and affect the nucleus in the anterior half shielded by lead, or to an indirect effect exerted on the nonirradiated nucleus by active substances produced by radiation in the treated cytoplasm.

Welshons, W. J., and Hinton, C. W. Bridges at anaphase II in ring-X males.

Braver and Blount (Genetics 35: 98, abstract, 1950) recorded the occurrence of anaphase bridges in larval neuroblasts of females heterozygous for either the  $X^{c2}$  or the unstable  $w^{vc}$  ring chromosome. Genetic analysis of the unstable  $w^{vc}$  chromosome (Hinton, in press) suggested that anaphase bridges might also be formed in males during the second meiotic divisions to produce either nullo-X-nullo-Y gametes or dominant-lethal gametes. Aceto orcein smear preparations of testes from  $X^{c2}$ , cv v f/Y and unstable  $w^{vc}/sc^8.Y$  males were examined. Two bridges were observed among 110 second-division figures from seven  $X^{c2}$  males, and 9 out of 104 second anaphases from thirteen  $w^{vc}$  males exhibited bridges; this corresponds to 3.6% and 17.3% for the bridge frequencies in  $X^{c2}$  and  $w^{vc}$  males, respectively, after correction for the presence of the Y chromosome in one-half the anaphases. Although a few bridges were clearly double dicentrics, no definite identifications were made of interlocked rings. These observations provided no indication of whether bridges formed at this division are lost or broken.

Weltman, A. S. Mortality and infertility effects of cold-shock treatments on *D. melanogaster* females.

refrigerator, rather than the deep-freeze unit employed by the first authors, to obtain similar desemination of fertilized females.

Our continued use of the deep-freeze technique revealed an unexplained change in the behavior of many stocks in the laboratory upon subjection to cold treatment. The excessively high mortality effects, and distinctive variations of many of the stocks rendered it desirable to test the mortality and infertility effects of cold-shock treatment on several of the stocks

An effective method of desemination of fertilized *D. melanogaster* females was outlined by Novitski and Rush (1949). Scossirola (1954) described the use of the freezer-locker compartment of an ordinary

maintained in the laboratory. Stocks were randomly selected. Some possessed markers on the first, second, third, or fourth chromosomes; one stock contained a double-X, one an attached-X, and others were heterozygous for inversions of the second or third chromosome.

Analysis showed that in the case of twelve stocks studied, the percentages of females remaining alive at a corresponding age level of 16-17 days for the control groups varied from 59.6% to 100.0% for the various stocks. The treatment periods emphasized the sensitivity of particular stocks to the cold-shock treatment; for when females were exposed to -10 degrees for 5 minutes, values of 2.5% to 85.0% were observed. The treatment period of 7 minutes gave a range of 2.6% to 60.0% females surviving after 16-17 days; and 10 minutes' treatment gave a range of from 0% to 35.0% surviving females. A similar situation was found when one compared the percentages of females that maintained their fertility after having been deseminated by the cold treatment. A close correlation was found, in that stocks showing high mortality effects likewise demonstrated high infertility effects as a result of the cold treatment. To illustrate the values observed: in Canton-S stock the percentages of females surviving were 84.4% for the control group, 7.7% for the 5-minute interval, 2.6% for the 7-minute period, and 0.0% for the 10-minute period; in Bar stock the corresponding values were 93.9%, 20.0%, 11.9% and 5.0%; Cy/fr<sup>2</sup> wt stock had values of 96.7%, 59.0%, 13.8%, and 0.0%; and ci ey<sup>R</sup> stock had 94.9%, 85.0%, 60.0%, and 10.0%.

Similar studies were made of seven lines obtained from fertilized D. melanogaster females trapped in a local fruit store. Whereas control values ranged from 81.4% to 100.0%, the percentages of survivors in the seven lines after treatment for 7 minutes ranged from 1.4% to 17.1%. The apparent similar reduction in the viability and fertility of the seven lines by the cold-shock treatment is attributed to similarity of genotype in these lines obtained from one source. The high sensitivity to cold shock might be attributed to the possibility that these lines were derived from flies of tropical and semitropical strains, carried into the store via fruit shipments.

The results are conclusive in showing that individual stocks have particular, inherent sensitivities to cold-shock treatment, which are attributable to their genotypes. Considerable differences in response with regard to percentage of survivors, and percentage of fertile females, have been demonstrated by the stocks analyzed. The extreme effects of cold shock upon some stocks renders it advisable to take this factor into account if one uses the procedure of desemination of fertilized females as a method to obtain virgin females. Further testing is required to find a temperature and a time interval at which mortality and infertility effects are minimized.

Weltman, A. S. The effect of inversions, the proximal piece of the B<sup>S</sup> translocation, and the Y chromosome on crossing over in attached-X females.

males except those bearing the Y chromosome were mated to y B, X-Y males; females bearing the y chromosome were mated to B males.

A comparison was made of the effects of autosomal inversions, the Y chromosome, and the proximal piece of the B<sup>S</sup> fragment on crossing over in attached-X females bearing the markers y v f on one arm, and sc cv on the other. All attached-X fe-

Type of female	Region y - v	Region v - f	Region f - s.f.a.	Total	Total no. females
XX/B <sup>S</sup> duplication	37.7	17.8	5.1	60.6	663
XX/Y	31.4	21.2	8.4	61.0	12,399
XX/0	36.1	21.8	11.3	69.4	49,626
XX/0; Ubx130	41.0	24.8	14.6	80.6	11,477
XX/0; Cy	42.6	25.1	13.3	81.1	12,799
XX/0; Ubx; Cy	56.9	30.5	23.0	110.5	12,948
Standard map dist.	33.0	23.7			

Comparison of the crossover values in the XX/0 type with the standard map distances shows an increase of 9.6% over the "expected" standard in the y-v region and a decrease of 7.6% in the v-f region. Presence of Ubx130 caused a total increase of 16.1% over that of the XX/0 type with the greatest percentage of increase, 22.4%, occurring in the region from f to the centromere. Cy, however, resulted in slightly greater increases of crossing over in the euchromatic regions y-v (17.9%) and v-f (15.0%) than did Ubx130. However, the latter caused a greater increase of crossing over in the region from f to the centromere. The presence of both inversions in the same female resulted in increases greater than the sum of the effects of Ubx130 and Cy independently; the greatest increase over that found in the XX/0 type, 103.5%, was observed in region f to the centromere.

As with unattached-X females, the presence of the Y chromosome and the proximal piece of the B<sup>S</sup> translocation, because of their homologies with the proximal region of the X chromosome, revealed a considerable inhibitory effect on crossing over in attached-X's in the region from f to the centromere.

Yaffe, D. An experiment on the enzymatic basis of the phenocopic effect produced by silver nitrate.

Flies grown on food containing silver nitrate exhibit a number of phenocopic modifications, especially the well known deficiency in melanin pigmentation, which has been compared to the mutation "yellow."

Since it is known that melanin is produced by an enzymatic system acting on tyrosine, it was the aim of the present experiment to test the effect of silver nitrate on this system *in vitro*.

If a prepupa is squashed in a drop of tyrosine solution (2:10,000) on a slide, the drop darkens rapidly under the influence of the hemolymph, much more so, in fact, than either tyrosine alone or a larva squashed in distilled water. When a pupa grown on silver nitrate medium is employed in this test, the darkening of the drop is much delayed. A better demonstration of this reaction can be obtained by a "pseudochromatographic" method. Single prepupae are squashed on a sheet of filter paper, Whatman No. 1, 15 mm above the lower margin. The paper is rolled into a cylinder and dipped into a tyrosine solution standing 5 mm high in a dish. The dish is introduced into a glass cylinder and the latter is sealed with vaseline. When larvae grown on normal medium are employed, a dark streak will develop above the start point as the tyrosine solution rises. The gray color deepens to blackish in the course of three hours and becomes even darker within one day. The spots remain unchanged after drying and the papers can be kept indefinitely for reference. In order to test the effect of silver nitrate on this color reaction, the paper is soaked in a 0.005% solution of silver nitrate and dried in the dark before squashing the larvae on the start points.

Results: A) After three hours the color reaction appears to be definitely inhibited on silver nitrate paper. In most cases the dark streak is absent; the area where it should appear is surrounded by a pinkish halo, apparently owing to some reaction of silver nitrate with the hemolymph. After twenty-four hours the streak has usually darkened to gray, but it is still much lighter than that of the control. It would appear, therefore, that the inhibition of the oxidative reaction by silver nitrate is not complete. Even though conditions are kept as constant as possible, some prepupae will produce darker streaks on silver nitrate paper than others. This variability may well be compared with the variability of the pigmentation of flies grown on silver nitrate.

B) Prepupae which have developed on medium containing 0.05%  $\text{AgNO}_3$  produce no streak at all or, occasionally, a very pale one, when squashed on untreated paper. These "pseudochromatograms" are practically identical with those of control larvae squashed on  $\text{AgNO}_3$  paper.

C) Prepupae of the mutant strain "yellow" squashed on untreated paper produce streaks as dark as those developed from wild-type larvae. This result is in agreement with the conclusions of Graubard (Jour. Genet. 27: 199-218), who demonstrated with a different method that the mutant "yellow" is not deficient in tyrosinase.

Conclusions: It appears that silver nitrate disturbs melanin formation by inhibiting one (tyrosinase?) or several enzymes concerned in the oxidation of tyrosine and its derivatives. This inhibition is paralleled by the known action of silver ions on potato tyrosinase, which is inactivated by Ag. While the phenocopic effect of silver thus depends on a blocking of this oxidative reaction, the mutant "yellow" appears to be disturbed in an entirely different step of the melanin-forming system.

Zimmering, S. Non-random disjunction in the  $V^5$  and Grape translocations in *Drosophila*.

Novitski (Genetics 36, 1951). The results from less extensive experiments with  $T(2;3)bwV^5$  and  $T(2;3)Gr$ , which would be expected to behave in a manner similar to that reported above, show that they do also. From the cross of  $V^5/stw^3\text{ c st}$  females by  $stw^3\text{ c st}$  males, the results obtained are as follows (complementary classes are given as non- $V^5/V^5$ , and for single crossover chromatids, the shorter/longer): non-c.o.: 5948/6521; c.o.  $stw^3\text{-c}$ : 805/334; c.o. c-st: 141/77. From crosses of  $Gr/stw^3$  females by  $stw^3$  males, the results were: non-c.o.:  $Gr-370$ ,  $stw^3-373$ ; c.o.: +97,  $Gr stw^3-25$ .

Zimmering, S. The effect of the Y chromosome and Y chromosome fragments on crossing over in the autosomes of *Drosophila*.

exceptional offspring.

It was previously reported (Zimmering, DIS-28) that the phenomenon of nonrandom disjunction of unequal-lengthed chromatids of an asymmetric dyad was found to operate in  $T(2;3)bwV^4$ , as had been predicted by

Females of the sex chromosome constitutions listed in the table below, and heterozygous for  $G1\text{ Sb}$  ( $G1\text{ Sb}/+$ ) were crossed by Canton-S males. Crossingover in the marked third chromosome interval was followed separately in regular and

<u>Constitution of female</u>		<u>N</u>		<u>% c.o.</u>	<u>Gl-Sb</u>		<u>% XX-Y</u>
	<u>Exc.</u>	<u>Reg.</u>		<u>Exc.</u>	<u>Reg.</u>		
+/+	-	1202	-	10.3 ± 0.9		-	-
sc dl-49 v B <sup>M1</sup> /y <sup>2</sup> w <sup>a</sup> v	-	1029	-	22.0 ± 1.3		-	-
sc dl-49 v B <sup>M1</sup> /y <sup>2</sup> w <sup>a</sup> v/Y	949	543	18.3 ± 1.3	17.9 ± 1.6	77.8 ± 1.1		
sc dl-49 v B <sup>M1</sup> /y <sup>2</sup> w <sup>a</sup> v/Y <sup>lc</sup>	517	512	24.0 ± 1.9	22.9 ± 1.9	66.8 ± 1.5		
sc dl-49 v B <sup>M1</sup> /y <sup>2</sup> w <sup>a</sup> v/Y <sup>s</sup>	210	1526	20.5 ± 2.8	20.6 ± 1.0	20.5 ± 1.0		

It may be concluded that a complete Y chromosome has no clear enhancing effect on crossingover in the Gl-Sb region when crossingover in the X chromosome is almost completely suppressed. If anything, the presence of a complete Y chromosome would appear to somewhat neutralize the effect of these X chromosome inversions, the effect being restored, to a lesser or greater degree, by the substitution of Y chromosome fragments for the complete Y chromosome. These results from females carrying a complete Y are unlike those reported by Schultz (C.S.H.S. 15, 1951) from ClB/+ and ClB/+/Y females in which crossingover in the D-Sb-H regions was followed: in the absence of a Y chromosome, the crossover value was  $24.0 \pm 0.77$ , and in the regular offspring of XXY females,  $33.0 \pm 2.74$ .

From XX and XXY females heterozygous for the small, distal X chromosome inversion, sc<sup>7</sup> (permitting a crossover rate in the X chromosome of about 75% that of normal), and having the second chromosome constitution b en/lt (b and en lie about 7 map units to the left and right, respectively, of lt, which lies at the centromere), the following results were obtained: in the absence of a Y chromosome,  $12.2 \pm 0.7\%$  crossover in the marked intervals in 2; in regular offspring from XXY females,  $11.0 \pm 0.5$ ; and in the exceptionals,  $12.9 \pm 1.24$ . Thus, in the absence of any major upset in the X chromosome the complete Y does not alter the crossover rate.

## TECHNICAL NOTES

Bateman, A. J. Another method for dominant lethal studies.

There are many medium recipes for use in dominant lethal studies. I consider this one to be best because it is very simple to apply. The medium consists of 2.5% agar, 2.5% fresh creamed yeast, 0.075% Nipagin (added in alcohol solution) and black treacle sufficient to produce a moderate brown color. Microscope slides, 3" x 1", are dipped into the hot medium to within one-half inch of the top to produce a thin coat of medium on one side. If the medium is thin, the eggs are laid on it instead of in it, leaving the surface level. The slides are placed diagonally in bunged 3 1/2" x 1 1/4" specimen tubes. Mated females are allowed to lay in the incubated tubes for 24 hours. Owing to the thinness of the medium, the atmosphere has to be kept saturated in order to keep it moist and to encourage generous and uniform egg laying. This is done by placing soaked towelling over the trays of tubes. Remove the towelling when ready to shake out the females. There will be sufficient moisture in the bungs to allow hatching to proceed. Twenty-four hours later the slides are examined on the stage of a low-power dissecting microscope. Though a longer interval is usually recommended, I have never found it to give an increase in hatching. If the larvae are numerous enough to obscure the eggs, place the tubes over a hot radiator just before counting. This will cause the larvae to crawl up the slide and off the medium.

Gomes, Fabio R. Use of 3/4-ounce creamers.

Restaurant-type 3/4-ounce creamers are cheap, durable, and easy to obtain. Plain caps with stapled pull-tabs may be obtained from any bottle cap company (Creamery Package Mfg. Co., 1243 W. Washington Blvd., Chicago 7) or caps with edge pull-tabs that leave the entire top surface free for writing may be obtained from Fill-Rite Corp., 353 Canal St., New York 13, N.Y. (ask for Dupli-Seal caps).

The following data indicate the effectiveness of creamers for single pair matings. A corn meal-oats-molasses-1% agar medium was used, pouring about 10 cc into each creamer and allowing to harden on a slant. Just before flies were introduced, a small piece of cellucotton was placed at the bottom edge of the agar slant and the surface seeded with dry, live yeast. One pair of wild-type flies was put in each of 30 creamers prepared in this way. Parents were removed on the 8th day and counts of progeny begun on the 10th day. Thereafter, offspring in each creamer was counted every other day until no more appeared. The following table gives the mean adult fly counts by two-day periods:

Days after mating	Mean number of offspring per creamer
10	23.0
12	68.9
14	33.9
16	14.0
18	7.4
20	22.4
22	31.3
24	14.2
26	10.4
28	4.2
30	0.0

The first generation (10th through 18th day) averaged  $147.7 \pm 9.49$  offspring per creamer with a normal frequency distribution. The second generation (20th day on) was strong but by the 24th day some of the creamers had begun to dry out. The results indicate that creamers with paper caps may be used for large scale experiments where 100-150 offspring are sufficient for determination of segregation results.

Hasset, Charles C. A simple method for collecting *Drosophila* eggs.

Eggs can be obtained in very large numbers with little effort by the use of laying pans filled with a stiff paste of pressed yeast and water. Stainless steel pans,

6 x 3 x 0.6 cm., are filled with the paste and put into clean half-pint milk bottles, in each of which there should be several hundred flies of suitable age. A few hours later the pans are removed; the yeast is then scooped out and stirred in a beaker of water. The mixture is poured through a 200 mesh wire screen to separate the eggs, which are retained in the sieve. In a single day as many as 50,000 eggs have been collected from ten bottles in this way.

If times larvae are desired, the eggs are resuspended in water and collected on a small filter paper disc, using a Buchner funnel to allow them to spread in a thin layer. The filter disc is then laid, eggs downward, over a layer of yeast paste in a Petri dish. The larvae can be removed at any stage in the same way. Larvae in different instars can be separated by the use of various sizes of sieves. Sixty mesh screening will retain third instar *D. melanogaster* eggs, and 80 mesh can be used to separate the second instar from the first. These separations must be done quickly, for larvae too large to go through a screen passively will squeeze through the meshes if given time. Because of size variations in each instar, absolute discrimination cannot be achieved, but proper timing and visual examination will assure a high degree of accuracy.

Kaplan, W. D., Ojima, Y., Tanaka, K., and Tanaka, T.  
A method for handling *Drosophila* eggs for cytological study.

suggested the following method.

For this technique mantle tissue of the garden snail, *Helix aspersa*, is required. The mantle need not come from this species of mollusc, but it is believed that any mantle tissue would serve the purpose. *Drosophila* eggs are aligned on pieces of mantle about 8 x 5 mm. and left there for five minutes. During that time the mantle cells secrete large amounts of mucus which serves to imbed the eggs and hold them in position. After five minutes the pieces of mantle are then immersed in fixative, and carried through the necessary steps of dehydration and imbedding in preparation for sectioning.

It is necessary, however, to choose a fixative which is suitable for providing good chromosome fixation and which will not dissolve the mucus that holds the eggs in position. For this purpose we have found Kahle's fixative highly satisfactory. The chorions of the eggs may easily be broken to permit penetration of the fixative shortly after the pieces of mantle have been immersed in the fixing solution. Reference to Kahle's fixative is given in *The Biology of Drosophila*, p. 66.

The handling of *Drosophila* eggs in preparation for cytological observation is frequently done in this laboratory. During a visit to the laboratory, Dr. Ojima observed some of the difficulties attendant upon handling these small objects and

Sang, James H. Synthetic  
medium for the sterile  
culture of *Drosophila*.

Although a number of synthetic media have been developed for the aseptic culture of *Drosophila*, it has long been clear that they do not contain only essential constituents in optimal supply.

The medium defined below was constructed from dose response curves for all the constituents listed, except the salts, and can be considered complete, in the sense just stated, for the pure line Ore-S strain used. Other strains may have different needs, particularly with respect to casein, RNA, cholesterol, fructose and possibly also lecithin levels; but they are unlikely to differ greatly from those listed. With the exception of folic acid, the amounts of vitamins scheduled below are about ten times the minimum required by Ore-S for normal growth, and are therefore unlikely to need adjustment for other strains. These excesses have no deleterious effect whereas excess folic acid tends to slow growth.

Agar	3.00 gm.	Thiamine	0.0002 gm.
Casein	5.50 gm.	Riboflavin	0.0010 gm.
Fructose	0.75 gm.	Nicotinic Acid	0.0012 gm.
Cholesterol	0.03 gm.	Ca pantothenate	0.0016 gm.
Lecithin	0.40 gm.	Pyridoxine	0.00025 gm.
RNA	0.30 gm.	Biotin	0.000016 gm.
NaHCO <sub>3</sub>	0.140 gm.	Folic Acid	0.00010 gm.
KH <sub>2</sub> PO <sub>4</sub>	0.183 gm.		
Na <sub>2</sub> HPO <sub>4</sub>	0.189 gm.	Water to 100 ml.	

Ore-S larvae pupate in about 4.8 days on this medium compared with 4.1 days when fed on an optimal supply of killed yeast. The greater part of this difference can be eliminated by adding Begg and Robertson's (1950) yeast fraction to the synthetic medium. Variability is about the same as on killed yeast.

Spieth, Herman T. Aniline  
marking dye for live *Drosophila*.

A modification of Dalmat's method of marking Simuliidae (Ann. Ent. Soc. Am. 43: 537-545) has been found effective for tagging living *D. persimilis* and *D.*

*pseudoobscura*. Each dye was prepared by thoroughly grinding 9 parts of refined wheat flour with one part of dye in a mortar. Methylene Blue and Neutral Red were used, but in addition, Dalmat has found Safranin O, Safranin Bluish, Victoria Green and Carmine effective for simuliids. A minute quantity of the mixture was placed in a shell vial and the living unetherized drosophilids were then introduced and shaken until they had come into contact with the dye powder. They were then returned to a rearing bottle and allowed to clean themselves. Such flies quickly appear normal in all respects and live just as long as untreated controls. Under a low power binocular microscope it is often possible to identify the dye used by inspecting the transparent wing membrane but positive identification can always be made by sacrificing the fly and immersing it into a drop of fluid consisting of three parts absolute alcohol, two parts glycerine, and one part chloroform. The stained flies always impart to the solution the characteristic color of the dye. The age of the fly when dyed and the length of time after staining does not in any way affect the ease of identification. Dalmat used this technique for determining flight ranges of simuliids while the present author has employed it to mark both females and males of the sibling species *pseudoobscura* and *persimilis* for multiple choice mating experiments. Pavan (personal communication) has effectively utilized the technique on other species of *Drosophila*.

Strømnaes, Ø., and Hannah,  
A. Mounting of Drosophila  
legs.

of dead flies and present a problem even when the flies have been freshly killed and the legs mounted in euparal before rigor mortis has set in. Etherizing the flies with acetic-ether instead of ethyl-ether tends to leave the legs somewhat more relaxed. If the flies are stored in alcohol, rigidity and particularly brittleness of the legs increases. Since it is not always convenient to make the mounts immediately after etherization, the authors have searched for a satisfactory method for mounting flies that have been stored in alcohol.

Recently a modification of a technique described by J. D. Corrington (Working with the Microscope) has been worked out, and has been found to give excellent results even after the flies had been stored for as long as five months in 96% alcohol, or two years in absolute alcohol.

The legs with their bases were dissected out usually by cutting them, as a group, from the thorax. They were incubated at 70° C. for one hour in a M/5 (11.34%) KOH solution. They were then rinsed thoroughly in water, to remove all KOH, and transferred through two changes of dioxan for one-half hour each, then mounted in balsam. This method gave legs which were flexible and easy to handle and consequently mounts with the legs in position and spread out well.

Other methods, such as boiling the legs for a short time in water after the KOH treatment, or dehydration with butyl- or ethyl-alcohol were tried. Legs so treated were easy to mount. For morphological studies, however, these methods were found to be less satisfactory since the legs had a tendency to shrink and to lose hairs.

Van der Kloot, W. G., and  
Levine, R. P. An electrical  
foot-focuser for the dis-  
secting microscope.

However, all of these mechanisms require skilled machining in their construction; therefore an electrical foot-focuser has been developed. Such a system must include a motor and gear system with a low speed, high torque output, and an electrical brake for precise stopping. These criteria are met by commercially available rotators for TV antennae. We have used a Crown model CAR-6A rotator (\$26.43) which makes one revolution per minute. A two position, teeter-totter foot switch was connected in the place of the hand switch in the rotator control box. The bracket for securing the antenna was removed with a hacksaw and the rotating wheel was coupled directly to the focusing knob of the microscope. If a faster rate of change of focus is desired, the coupling could be made through a step-up pair of gears.

Wheeler, M. R. A dry  
ingredient medium for  
field collectors.

The dry ingredients are premixed in the laboratory and packaged in batches adequate for about 50 vials per batch. To use, the collector boils the ingre-

Preparation of the legs of *Drosophila* for morphological studies has proven to be very difficult because of their rigidity and brittleness. Stiff legs are typical

It is frequently useful to employ a dissecting microscope equipped with a foot-focusing attachment so that both hands are left free. For this purpose various mechanical systems have been described.

This medium was designed to simplify the food problem of field collectors and to permit the shipment of live flies back to the laboratory with a minimum of trouble.

dients in a measured amount of water and pours directly into vials (no autoclaving is necessary). The surface is smooth and dry, growth of bacteria and mold is strongly inhibited, and larvae will grow satisfactorily, though slowly, in it.

table sugar	9 gm.
agar, granulated	5
yeast, dry dead	5
moldex, powdered	0.3
$\text{KH}_2\text{PO}_4$	1.5

The required amount of water for one batch is 250 cc., or one-half pint, or one measuring cup. One batch makes 50 vials when poured to a depth of about one-half inch per vial; more food per vial is not necessary for mailing purposes.

Wheeler, M. R. An improved *Drosophila* collecting net.

Basic idea: a clear unbreakable plastic vial is attached to the tip of the net so that flies accumulate in it as the net is being used; then a cotton plug is quickly inserted into the open end of the vial and the vial with its contents is removed from the net. A fresh vial is then inserted into the net for further collecting.

Materials needed: any standard insect net; plastic vials (I use Turtox No. 130A26, size C, with threaded top); plastic (polyethylene) funnel; glue.

Construction: both top and bottom of the funnel are cut off with a sharp knife and discarded, leaving a one-inch center section; the bottom cut is made very carefully so that a plastic vial will just fit into this opening. The outer surface of the funnel piece is roughened (sandpaper, etc.) so that it will bond firmly with glue, and it is then glued into the tip of the net. The netting remaining over the open end of the funnel piece is cut away with scissors and the cut edge glued. The plastic vials are heated briefly to soften the open ends which are then flared outwards by pressing against some rounded surface. Thus when the vial is inserted through the open end of the funnel and pulled tightly into place, the flared edge fits against the funnel surface and the threads of the vial make a firm grip with the edge of the funnel opening. I also modify the net by shortening it and by increasing the taper toward the tip.

Advantages: one can see at any time how many flies have been caught; removal of caught flies by plugging the vial in place prevents their escaping; if a collecting vial becomes dirty, wet, etc., it takes less than a minute to remove it and insert a clean one. Finally, the vial size given above (size C) is so nearly the same as that of our standard shell vials used in the laboratory that transfer of flies from one to the other is not difficult.

## TEACHING NOTES

Burdick, A. B. Monohybrid for sophisticated students.

One of our Bar stocks has two lethals on the first chromosome with Bar. These lethals, for reasons unknown, stay in the stock in high frequency and provide an interesting experiment in our advanced laboratory course. At the beginning of the semester we propose a sex-linked monohybrid involving Bar as the first experiment. Although this is quite beneath many of the students, the results challenge all of them for interpretation. We ask them to make five single pair matings of  $B/B\varphi \times +/\delta$ . With the two lethals in the stock, these matings may be of several types:

1) $\underline{l^1} B/B \times +/\delta$	gives	2 wide Bar $\varphi\varphi$ : 1 Bar $\delta$
2) $B/B \underline{l^2} \times +/\delta$	gives	2 wide Bar $\varphi\varphi$ : 1 Bar $\delta$
3) $\underline{l^1} B \underline{l^2}/B \times +/\delta$	gives	ca. 3 wide Bar $\varphi\varphi$ : 1 Bar $\delta$
4) $B/B \times +/\delta$	gives	1 wide Bar $\varphi$ : 1 Bar $\delta$

We also get some matings that appear to involve  $\underline{l^1} B/B \underline{l^2}$  females which indicates that one of these "lethals" must, occasionally, go through the male.

The data of any given student usually contains one or more bottles with aberrant sex ratios. They find heterogeneity with Chi-Square and, if they have only 2:1 and 1:1 ratios, usually come up with a lethal in their interpretation. But, when 3:1 (and sometimes 10:1) ratios appear, they seem to lose confidence in lethals especially when they consider how a female could get to be  $\underline{l^1} B/B \underline{l^2}$  to give a ca. 10:1 ratio. I feel they learn quite a bit about the critical handling of data from this experiment. It evokes considerable discussion and frequently leads to interesting interpretations.

Burdick, A. B. Xasta in class experiments.

Good, clear, uncomplicated translocation segregation data is difficult to obtain in *Drosophila*. Most translocations are not well marked and multichromosomal recessive testers frequently conflict in expression with translocation markers. One combination that has worked well for us is  $T(2;3)Xa$  with  $m;cn;e$ . We cross:  $m;cn;e\varphi \times T(2;3)Xa/Ubx^{130} \delta$  and backcross:  $m/+; cn-e/T(2;3)Xa\varphi \times m;cn;e\delta$

Typical backcross data are those of Mr. V. M. Sahni, last year:

	<u>♂♂</u>	<u>♀♀</u>
Xa	34	53
(Xa)m	44	44
m, cn, e	29	41
cn, e	35	44

We never obtain any recombination between *cn* and *e* which is unfortunately due to the fact that *Xa* also carries *In(2R)Cy*, which covers *cn*, and *In(3R)P* with a break near *e*. This prevents location of the break-points of *Xa* from the data and leads everyone to conclude that the breaks must be very close to both *cn* and *e*. I think the recessive tester stock could be improved by making it *B;cl;ss<sup>a</sup>* which should give some recombination between *cl*, *ss<sup>a</sup>*, and *Xa* to allow genetic estimation of the break-points.

Goldschmidt, Elisabeth.  
Phenocopy and species  
hybrid in class work.  
can be ensured.

The following simple experiments are familiar to geneticists from the literature, but it is not generally realized how easily their success in class work

a) "Yellow" phenocopy on silver nitrate medium. Medium containing 0.1%, 0.05% and 0.025%  $\text{AgNO}_3$ , respectively, is prepared by stirring appropriate volumes of a 5%  $\text{AgNO}_3$  solution into standard food mixture cooled to 60° C. directly after boiling. Since stocks vary in their sensitivity to the salt, the concentration which produces a high percentage of phenocopies (50-80%) without being too toxic to the flies, is determined each year by a test series before starting the course. At least 10 pairs of parents should be introduced into each half-pint bottle. Students test different isogenic strains (or different *Drosophila* species) on the same medium or one stock on a series of concentrations. Light-colored flies are transferred to normal medium to demonstrate noninheritability of the effect in their offspring.

b) Hybrid *D. melanogaster* x *D. simulans*. Virgin males and females, aged for different periods, are shaken without etherizing into "creamers," according to the method described by Uphoff (Genetics 34: 314-327). Thirty to fifty per cent of students' crosses prepared in this way are fertile. Hybrid larvae are utilized for salivary preparations to demonstrate the inversion constituting the main cytological difference between the parent species. Adult female hybrids are tested for sterility. Change of dominance relations in the hybrid can be demonstrated by utilizing *melanogaster* females carrying dominant genes. Thus, when employing Cy L/Pm females, Cy is found to be dominant, while L and Pm are recessive in the hybrids.

#### NOMENCLATURE

Frydenberg, Ove. Notes  
on nomenclature.

The type specimens of several critical *Drosophila* species have been investigated and it has been revealed that *D. pleurofasciata* Duda 1924, and *D. macularis* Villeneuve 1921 are invalid synonyms of *D. picta* Zetterstedt. It was also shown that *D. vibrissina* Duda 1924 and *D. grischuna* Burla 1950 are invalid synonyms of *D. confusa* Staeger 1844. The latter, however, is specifically different from *D. funebris* Fabricius.

#### MATERIALS REQUESTED OR AVAILABLE

E. B. Basden (Institute of Animal Genetics, Edinburgh, Scotland) would like live wild-caught adults of *Parascaptomyza disticha* (= "Scaptomyza graminum") -- the species with only two rows acrostichals -- from anywhere in the world (see Research Notes).

K. W. Cooper (University of Rochester, Rochester, New York) would appreciate receiving any free duplications occurring in *D. melanogaster*, or any

## RESEARCH NOTES

Barigozzi, C. Mitoses in differentiated cells of D. melanogaster.

this, mitoses have been observed, showing chromosomes two to four times bigger than in neuroblast mitoses. The different pairs are easily classifiable, although synapsis is very strong.

Barigozzi, C., and Di Pasquale, A. Localization of genes controlling pseudotumor production in *Drosophila*.

on all pairs. The present report deals with an attempt to localize tu genes, in one stock, with the second chromosome, which is practically the only site of tu genes in some stocks. Using as markers cn c px, crossing over proved the existence of more than one tu locus, probably located on both sides of px. Thus, tu genes are a system of genes, acting as polygenes, restricted to a short portion of one single chromosome. The presence of modifiers can be proved by means of selection. Starting with stocks that are practically homozygous for tu genes, it is possible to select both for increasing and for decreasing tumor frequency. Using this technique, it is possible in some cases to raise the frequency up to nearly 100% or to lower it down to 3% or less. This is done merely by changing the modifier assortment. Thus, crosses between + and - selected lines give a high pseudotumor frequency because the recessive tu's are still present in both lines. In the stocks investigated so far we thus find two systems of polygenes, one restricted to a short chromosome portion, and the other (the modifiers) spread throughout the whole genome. The degree of homeostasis in different stocks, detected through interruption of selection, proves that carrying pseudotumors can be semilethal or deleterious in some stocks, but harmless and even advantageous in some others. No generalization can be made.

Becker, H. J. On X-ray-induced somatic crossing over.

imagines were checked for single (w/w or w<sup>co</sup>/w<sup>co</sup>) and twin mosaic spots. The factor sn was not immediately significant in this connection; se was used in order to increase the color contrasts, and h for easier recognition of the se chromosome. In this experiment (inspection of the imagines first in air and afterwards under parafin oil), 336 twin spots, and 115 white and 32 dark single spots were found. In a second experiment (only larvae of the first and second instar treated; inspection of the imagines in air; complete formula, see below), 1225 twin spots, and 203 white and 156 dark single spots were obtained. The different frequencies of white and dark single spots are probably due to the relative difficulty of distinguishing the dark spots, especially in older imagines. The discrepancy between the percentages of twin spots in the two experiments is probably due to the fact that in the first experiment younger stages were treated. Twin spots induced during these stages are more often situated, by reason of their size, with only one partner in the eye, and are thus counted as a single spot.

Migrant hemocytes are cells which have already undergone histological differentiation. This is proved by the low polyteny of their nuclei. In spite of

showing chromosomes two to four times bigger than in neuroblast mitoses. The different pairs are easily classifiable, although synapsis is very strong.

Previous investigations have proved that two different parts of the genome are at work in producing pseudotumors in some stocks of D. melanogaster: tu genes on the second chromosome, and many modifiers

on all pairs. The present report deals with an attempt to localize tu genes, in one stock, with the second chromosome, which is practically the only site of tu genes in some stocks. Using as markers cn c px, crossing over proved the existence of more than one tu locus, probably located on both sides of px. Thus, tu genes are a system of genes, acting as polygenes, restricted to a short portion of one single chromosome. The presence of modifiers can be proved by means of selection. Starting with stocks that are practically homozygous for tu genes, it is possible to select both for increasing and for decreasing tumor frequency. Using this technique, it is possible in some cases to raise the frequency up to nearly 100% or to lower it down to 3% or less. This is done merely by changing the modifier assortment. Thus, crosses between + and - selected lines give a high pseudotumor frequency because the recessive tu's are still present in both lines. In the stocks investigated so far we thus find two systems of polygenes, one restricted to a short chromosome portion, and the other (the modifiers) spread throughout the whole genome. The degree of homeostasis in different stocks, detected through interruption of selection, proves that carrying pseudotumors can be semilethal or deleterious in some stocks, but harmless and even advantageous in some others. No generalization can be made.

Embryos and larvae of constitution w sn<sup>+</sup>/w<sup>co</sup> sn; se h/se h were X-rayed with a dose of 1200 r, and the eyes of the

imagines were checked for single (w/w or w<sup>co</sup>/w<sup>co</sup>) and twin mosaic spots. The factor sn was not immediately significant in this connection; se was used in order to increase the color contrasts, and h for easier recognition of the se chromosome. In this experiment (inspection of the imagines first in air and afterwards under parafin oil), 336 twin spots, and 115 white and 32 dark single spots were found. In a second experiment (only larvae of the first and second instar treated; inspection of the imagines in air; complete formula, see below), 1225 twin spots, and 203 white and 156 dark single spots were obtained. The different frequencies of white and dark single spots are probably due to the relative difficulty of distinguishing the dark spots, especially in older imagines. The discrepancy between the percentages of twin spots in the two experiments is probably due to the fact that in the first experiment younger stages were treated. Twin spots induced during these stages are more often situated, by reason of their size, with only one partner in the eye, and are thus counted as a single spot.

Other data contribute to the problem of the primary X-ray effect resulting in somatic crossing over. At the end of the first larval instar the presumptive eye area in the head anlage consists of about 20 cells (see the next note). After treatment of larvae at this stage, about 30 spots resulting from somatic crossing over were found among 100 eyes, that is, 30 spots per 2000 treated cells, or 1.5 spots per 100 cells. However, a mosaic spot arises as a consequence of somatic crossing over only when the two crossover chromatids join different cells, not when they join the same cell. Both these types of segregation occur with equal probability. Thus, not taking into account special double and multiple exchanges that may also result in mosaic spots, crossover events in the X chromosome take place in about 3 out of 100 cells. Fano and Demerec, after treating *Drosophila* sperm with 400 r, found 1% dominant lethals due to single breaks in the X chromosome--that is, 3% with 1200 r; thus two breaks, one in each chromatid, are to be expected in 0.09% of the cells and at homologous points with a disproportionately lower frequency. Since in somatic cells values of a similar order are to be expected, a two-hit event cannot be the cause of somatic crossing over. Since Friesen (1937) and Shapiro (1941) found a deviation from a linear dose-frequency relation for X-ray-induced mosaic spots, it does not appear that a single-hit event, either, is the cause of somatic crossing over. Lefevre's (1947) finding that the spot frequency depends upon the dose rate points in the same direction.

Since somatic crossing over thus hardly seems to arise through chromosomal breaks as a consequence of ionization, an attempt was made to get further information about its origin. The larvae of the second experiment were made heterozygous for the factor *rug* (3-0.0); thus the complete formula was  $w^{co}$  sn/w sn<sup>+</sup>; *ru*<sup>+</sup> se h/*ru* se h. Five twin spots were found in which the white partner showed the *ru* phenotype; the same phenotype was shown by the dark partner of 8 other twin spots, and by 4 white and 6 dark single spots. In addition the following 8 spots were found: (1) both twin-spot partners *ru*; (2 and 3) only a part of the white partner *ru*; (4) only a part of the dark partner *ru*; (5) inside a large *ru* region, a smaller twin spot; (6) inside a large *ru* region, a smaller white single spot; (7 and 8) inside a large *ru* region, a smaller dark single spot. The single color spots in (6)-(8) lie on the margin of the eye.

Provided that at least some of these types of spots are due to induced somatic crossing over in both the marked chromosomes, then these cases indicate that the X chromosome (in the case of the smaller color spots) and the 3rd chromosome (in the case of the smaller *ru* spots) did not undergo crossing over immediately at the time of treatment, but only later on. This would mean that not breakage events caused by ionization, but rather a physiological change in the cells, is responsible for the origin of somatic crossing over. This change may provide the conditions for crossing over, and may sometimes be maintained for one or more cell divisions after the treatment.

Becker, H. J. On the development of the *Drosophila* eye.

After X-ray treatment of  $w$  sn<sup>+</sup>/ $w^{co}$  sn; se h/se h embryos and larvae, twin mosaic spots are to be found in the eyes of the imagines. These spots are caused by somatic crossing over and consist each of a white *w/w* and a dark  $w^{co}/w^{co}$  partner (see the preceding note). The partners of spots induced before the end of the first larval instar in the lower half of the eye (this portion shows the clearest

After X-ray treatment of  $w$  sn<sup>+</sup>/ $w^{co}$  sn; se h/se h embryos and larvae, twin mosaic spots are to be found in the

conditions) are situated in a row, with the approximate direction, frontal-dorsal to caudal-ventral; in later-induced spots they are situated in a direction vertical to the former. The relative position of the two partners of the spots shows the direction of cell division of their common stem-cells. Thus at the end of the first larval instar a change takes place in the direction of the divisions.

This stage is further characterized by another phenomenon. Whereas spots induced before this time, that is, larger ones, have a random position within the eye, spots induced during this time occupy certain distinct sectors of the eye. It can be concluded, therefore, that the cells before this stage are not predestined to develop into distinct parts of the eye.

A third characteristic of the end of the first larval instar is that twin spots, having a total size of one of the above-mentioned sectors, are constituted of partners, the smaller of which is situated in the posterior middle of the eye. This situation is not found in the large, earlier-induced spots. The regular size differences in twin-spot partners show that a pattern of division intensity is built up.

It seems plausible that all three changes in condition are due to a uniform determination process, and that the designation of a number of cells of the head anlage as an eye anlage is connected with a change of orientation of the cell divisions and the creation of a pattern of division intensity. The total number of cells of the presumptive eye area is 18 to 20 at the time of determination, as calculated from the size of mosaic spots induced at the end of the first larval instar.

These findings were amplified by the analysis of a new mutant strain, in which the eyes of 35% of flies are normal at 18° C. In the other 65%, parts of the lower half of the eye do not form any ommatidia, but normal head cuticle instead. These parts are of the same shape as the X-ray-induced mosaic spots. They can be as large as a spot induced at the end of the first larval instar, or larger; the largest defect is the absence of the lower half of the eye. In spite of the different sizes of the defects, the peculiarities of the frequency distribution of the defective areas are best interpreted by supposing that the mutational effect is due to an interference with a process which gives certain cells of the head anlage the capacity for ommatidia formation. According to the size of the smallest defects, this process takes place at the stage when the presumptive eye anlage consists of about 18-20 cells. Obviously, this process is concurrent with the above-inferred determination of eye formation. These processes can be denoted as the creation of an ommatidia field.

In X-ray experiments with  $+\text{w}$  sn heterozygotes, spots with singed bristles occurred close to white ommatidia on the margin of the eye. The stem-cells of these spots were found in considerable numbers up to the end of the second larval instar. This shows that within the field determination the outline of the imaginal eye is not definitely fixed.

After treatment of larvae of the third instar, irregularities of the facet pattern of the eye were observed. The position of the irregularities is dependent on the time of irradiation. The sensitive zone migrates over the anlage during the third instar in a posterior-anterior direction. Possibly this phenomenon is connected with the definitive establishment of the outline of the eye, which takes place during the third larval instar according to investigations made by several authors with the mutant Bar.

Belitz, H. J. The distribution of mutations induced by 2:5-bis-ethylene-imino-benzochinone-1:4 on the genetical map of the X chromosome of D. melanogaster.

In experiments with the compound 2:5-bis-ethylene-imino-benzochinone-1:4 (Chinon I, Bayer G 4073), which yielded a mutation rate of 5.5% lethals (H. Lüers, 1956), all lethals and visibles were localized by means of a strain marked by sc ec ct v g f. Fourteen lethals (10.4%) were connected with gross structural changes of the sex chromosome. The distribution of the other 120 mutations on the genetical map of the X chromosome was compared with the distribution of 90 point mutations, 34 of which were obtained from different controls, the rest from negative mutation experiments. All mutations had arisen in the stock "Berlin wild" and were detected by the M-5 technique. As shown in the table, the relatively low mutation rate in the region sc-ec in the experiment with the chinone derivative is significant.

Region of chrom.	sc-ec	ec-ct	ct-v	v-g	g-f	f-sp-f	Total
n	30	14	9	14	14	9	90
Spontaneous	33.3	15.5	10.0	15.5	15.5	10.0	
%							
n	17	20	21	12	25	25	120
Chinon I	14.2	16.7	17.5	10.0	20.8	20.8	
%							
$\chi^2$	10.82	0.04	2.36	1.35	0.94	4.50	20.01

Bell, A. E. On the significance of fourth-chromosome polygenes in quantitative genetic studies with D. melanogaster.

The assumption of random distribution of polygenes among the various chromosomes is usually made in quantitative genetic studies. If one accepts this assumption, the need for genetic markers for the

fourth chromosome of D. melanogaster must be considered in the utilization of marked inversion stocks. While transferring the recessive fourth-chromosome gene pol, into a large-body-size stock as well as a small-body-size stock, some evidence was found which suggests that fourth-chromosome genes did not contribute to the genetic variation observed among these lines.

These large-and small-body-size lines were initiated from a common wild population and had been selected over a period of twenty generations for large and small body weight, respectively. For a related experiment, it was decided to insert the recessive marker, pol, into each of these lines. Males from a stock homozygous pol/pol and possessing dominant markers on the three major pairs of chromosomes were mated to virgin females of each body-size line. The  $F_1$  male progeny were then backcrossed to virgin females from the parental size line. Among the segregating progeny those classified as wild type possessed chromosomes 1, 2, and 3 exclusively from the original body-size line and undisturbed by recombination. As for the fourth chromosome, these wild-type segregants would consist of  $1/2$  pol/+ :  $1/2$  +/+. where the + chromosomes would be in toto from the original body-size line. From matings among these wild-type segregants, selection for body size was reinstated in the same direction as previously practiced. Over a period of three generations the following two phenotypic classes were classified and weighed within each body-size line: (1) polished (pol/pol), containing no fourth-chromosome genes from the original body-size line except for the possibility of limited

recombination in females; and (2) wild type, consisting of  $pol/+$  and  $+/+$  and possessing either one or two fourth chromosomes from the original body-size line. Thus, within each body-size line, a comparison is provided between the  $pol$  chromosome (unselected for body size) and the  $+$  fourth chromosome (selected for body size for 20 generations) when expressed against a common genetic background on chromosomes 1, 2, and 3. The results were consistent for all three generations and are summarized in the accompanying table.

Body weights in selected lines of D. melanogaster where classes differed only in their fourth chromosome

Body-size line	Average body weights (micrograms)			
	Males		Females	
	polished	+	polished	+
Large	(650)* 1,058.0	(333) 1,023.3	(633) 1,426.9	(328) 1,397.1
Small	(804) 618.3	(363) 612.0	(855) 844.6	(370) 848.6

\* Number weighed per class.

Comparing polished versus  $+$  within sex and within line, the obvious conclusion is that fourth chromosomes exposed to twenty generations of selection for large or small body size did not differ from the unselected polished chromosome in their influence on body weight. Since comparisons were made with both large and small lines, one cannot attribute the results to the possibility that the polished chromosome possesses unusually large or small body-size genes.

If further studies show the above-described situation to be general for other populations and other quantitative traits, efforts to genetically mark the fourth chromosome in D. melanogaster hardly seem justified in quantitative genetic studies, unless one wished to check the validity of assuming random distribution of polygenes.

Braver, G. Crossing over in the distal euchromatin of homozygous  $In(1)rst^3$ .

$In(1)rst^3$  (Gruneberg), with one break near  $rst$  (1.7) and the other in the proximal heterochromatin of the X chromosome, has a small distal noninverted euchromatic region. Using  $In(1)rst^3, w$ , produced by Novitski by irradiating  $In(1)rst^3$ , and kindly supplied by Lefevre, tests were made of exchange in the  $y-w$  region of  $rst^3/rst^3$  females ( $25^\circ C$ ). This region includes most of the distal euchromatin. Three crosses, made during the past year, gave crossover percentages of 5.5 ( $N=4624$ ), 6.6 ( $N=2165$ ) and 4.4 ( $N=6283$ ). In the first two tests, crossovers in the  $w$ -car region were detectable in the females. Values obtained were 14.6 ( $N=2425$ ) and 10.8 ( $N=1093$ ). An additional cross, in which the  $w$ -car and car-f regions were tested, gave values of 10.8 and 10.1 respectively ( $N=5296$ ). Salivary-gland studies of the homozygous  $rst^3$  females showed no chromosomal aberrations of X chromosomes or autosomes (other than the homozygous inversion), and crossover values for the  $y$ -car and car-f regions were comparable to those reported by Gruneberg (1935) and Mather (1938) for this chromosome. Crosses are now in progress in order to insert  $br$  (0.6) in one case, and  $pn$  (0.8) in another, on the  $rst^3$  chromosome. These markers will permit a more detailed study of the increased exchange in this distal euchromatic segment.

Brosseau, G. E., Jr. The recovery of detachments from a reversed acrocentric compound X chromosome.

In an experiment set up for other purposes, an unusually high frequency of detachments was recovered from Muller's double X, a reversed acrocentric.

Because of the reputed high stability of this compound and the unusual circumstances surrounding the recovery of the detachments, these data are particularly interesting. Virgin y f:± females were mass-mated to  $X^{c2}/Y$  males that had previously received 3300 r of X-rays. Among the progeny, 5528 attached-X chromosomes and chromosome products were recovered. Among these chromosome products were five y f males and one + female. The y f males must represent recovered detachments, but the female could just as well have been a superfemale or a triploid. Three of the five males were tested for fertility and found to be sterile. No actual control crosses were carried out, but no detachments were found among an estimated 2700 y f:± females from the stock cultures that provided the material for the X-ray experiment. The frequency of recovery in this experiment is about one detachment per 1000 compound X chromosomes. This must represent a minimum estimate of the detachment frequency, because one of the possible detachment products carries a lethal deficiency. The fact that most, if not all, of the detachments were recovered as males and the fact that these males were sterile suggest involvement of the paternal Y chromosome. Reversed acrocentrics give rise to second-anaphase bridges, which presumably break, yielding open ends that would most likely result in dominant lethality. The introduction of X-ray-fragmented chromosomes from the male might provide chromosome ends to cap these open ends and give rise to viable detachments. This problem is under further investigation with a different reversed acrocentric that is free of the complications in Muller's double X.

Brown, William P. A study of the mutant daughterless in a large laboratory population.

Since the discovery and description of the mutant daughterless, da (Genetics 39: 958-959), several interesting aspects of the mutant have been considered. One of

these is the selective advantage which da may or may not exhibit in a large population. The experiment presented here was an attempt to determine the activity of the mutant in a large random-mating population. A population cage was used to perform the study. The cage was kept in a room that had a temperature range of  $76^{\circ} F \pm 4^{\circ}$ . Fresh food was added twice weekly to the cage. The population was initiated by crossing wild-type virgin females obtained from a laboratory stock and homozygous da males. From the resulting progeny, 50 flies of each sex were etherized and introduced into the cage. By this procedure the initial gene frequency of da was .50. The presence of daughterless can be determined only by the progeny-testing of females. Those females which are homozygous for da, although wild type in appearance, produce normal sons but no daughters, regardless of the genotype of their mate. Eggs were collected from the population cage at weekly intervals, and a sample of 120 nonvirgin females hatched from these eggs were placed individually in food containers to produce progeny. The progeny in the successful cultures were observed and classified as to the presence or absence of female offspring, and the observation was recorded. In only one culture bottle, during the entire period, was the number of progeny so small that no definite classification could be made of the genotype of the female parent. The frequency of da for females in the population was calculated directly by application of the Hardy-Weinberg Law. The weekly gene-frequency values for the mutant are presented below. This 14-week period is equivalent to approximately 10 generations under conditions at this laboratory.

Weeks in cage	Successful cultures	No. da/da females	Per cent. da/da	da for females
0	-	-	-	.50
1	103	20	19.4	.44
2	111	11	9.9	.32
3	113	6	5.3	.23
4	105	1	1.0	.10
5	111	9	8.1	.28
6	111	6	5.4	.23
7	110	9	8.2	.29
8	111	9	8.1	.28
9	106	11	10.4	.32
10	115	5	4.3	.21
11	110	5	4.5	.21
12	112	7	6.2	.25
13	115	6	5.2	.23
14	113	8	7.1	.27

Tentatively, the mutant appears to exhibit some degree of selective advantage in a large random-mating population. It will be necessary to gather additional evidence over subsequent generations before reaching a conclusion as to the equilibrium frequency of this mutant.

Burdette, Walter J. Isolation of ecdysone from *Drosophila*.

Hormonal control of metamorphosis is easily demonstrated by ligation during the larval stage or by genic alteration of the ring gland. Crude hormone capable of inducing pupation was isolated from the Oregon-R stock raised in large quantity at 25° C on yeast-enriched medium. Collections of pupae, along with a small number of larvae in the late third instar, were made at the onset of pupation, after larvae had migrated to the walls of the containers in which they were cultured. This material was preserved in 100% methanol, and the extraction described by Butenandt and Karlson was employed for separation of the active hormone. Parallel extractions of silkworm chrysalises were also carried out. The yield from *Drosophila* (.018%) was greater per gram dry weight than that from dried silkworm chrysalises (.002%). However, the activity of hormone (in Calliphora units) from fresh material was less for *Drosophila* than for *Bombyx*. The total amount of crude hormone obtained from *Drosophila* was 111.2 mg from 6015.4 cc volume of fresh material, representing the total collection in the laboratory for one year.

Yield of hormone

Extrac- tion	Drosophila			Bombyx		
	Wt. of crude hormone (g)	Dry wt. of pupae (g)	% yield	Wt. of crude hormone (g)	Wt. of dried chrysalises (g)	% yield
1	.0246	87.3	.028	.0670	2520.0	.003
2	.0211	125.8	.017	.0287	1719.2	.002
3	.0175	134.6	.013	.0142	1112.2	.001
4	.0045	112.2	.004	--	--	--
5	.0436	171.1	.025	--	--	--
Total	.1113	631.0	.018	.1099	5351.4	.002

Burdette, Walter J. Lethal mutation rate after injection of water-soluble carcinogenic hydrocarbons.

Extensive testing with methylcholanthrene failed to give any indication that it increased the mutation rate of lethals on the X chromosome in D. melanogaster, even though tumor incidence was increased concomitantly in certain experiments. In a smaller number of tests, 1,2,5,6-dibenzanthracene also was not found to be mutagenic. Additional studies were carried out to determine whether these hydrocarbons yielded negative results because they are not soluble in aqueous solution. Mutation rate in the st sr e<sup>s</sup> ro ca; tu<sup>36a</sup> strain was tested by the Muller-5 method after males were injected at three days of age with 0.1% aqueous solution of sodium 1,2,5,6-dibenzanthracene-9,10-endo- $\alpha$ , $\beta$ -succinate. This experiment was then repeated, using sodium 3-methylcholanthrene-6,12b-endo- $\alpha$ , $\beta$ -succinate. Lethal mutation rates on the X chromosome were 0.21% with the former and 0% with the latter. Since the control rate of mutation was 0.08% in this strain, no evidence was obtained that these water-soluble compounds are mutagenic in *Drosophila*.

Carcinogen	No. of lethals	Chromosomes tested	Percentage lethals	P
Sodium 1,2,5,6-di-benzanthracene-9,10-endo- $\alpha$ , $\beta$ -succinate	2	965	0.21	0.34
Sodium 3-methyl-cholanthrene-6,12b-endo- $\alpha$ , $\beta$ -succinate	0	787	0.00	0.40
Control	1	1188	0.08	

Burdick, A. B., and Mukai, T. Viability of heterozygotes for l(2)55i.

Lethal l(2)55i occurs in the W-1(Erie) wild stock with  $q = 0.17 \pm$ , and appears to have maintained this level for over a year. It is located at about 51 on the second chromosome and is not associated with any crossing-over inhibition. When extracted with isogenic first, third, and fourth chromosomes and made heterozygous with a single (homozygous viable) second chromosome from W-1, l(2)55i remains in the population with  $q = .25$  after 16 random-mating generations. Our anticipated  $q$  for a recessive lethal in generation  $n$  being  $1/(n + 1)$ , leads us to expect a frequency of .06 after 16 generations, the difference (.25 - .06) apparently being due to the extraordinary viability of the lethal heterozygote. In addition, when l(2)55i, with its associated W-1 genome is made heterozygous with a single, unrelated genome from W-11, it may be found in frequency .29 after five generations when it is expected to be about .17. We determine the lethal frequency in each generation of these two populations and expect to continue to do so until a steady frequency is reached, at which time we expect to re-extract the populations.

Carlson, Elof A. Relocalization of the mutant rotund in the third chromosome.

The mutant *rn* (rotund) was reported by Glass (DIS-2: 8, 1934) to be of probable X-ray origin and within 1.6 units of *B1* (Bristle). Bridges, using *hk* (hook) and *B1*, thought it to lie between these loci (DIS-9: 85, 1938), but Muller found *rn* to be to the right of *B1*, between *B1* and *tk* (thick). More recently Muller (DIS-27: 106-107, 1953) reported that an irradiated stock of *rn*, selected for crossover suppression in the second chromosome, also contained "a large scale translocation with chromosome 3," which he designated as *rn*, T23. This *rn*, T23 was associated with a high degree of autosomal nondisjunction. According to Muller the original *rn* stocks were likely to have contained this translocation. In the same year Oksala (Proc. 9th Intern. Congr. Genet. II: 789) also noted the peculiar pairing relations of *rn*, which he localized between *lt* (light) and *rl* (rolled), concluding that *rn* coincides with the centromere, which he referred to as a "rotund-centromere."

In a stock of *sc<sup>L6</sup>*, a scute, rotund male was observed. Since this *rn<sup>2</sup>* male (see New Mutants, this issue) was sterile, several of his *sc<sup>L6</sup>* brothers were mated to *fes pr rn*, *T23/al<sup>2</sup> Cy cn<sup>2</sup> L<sup>4</sup> sp<sup>2</sup>* females. From a line showing *rn* offspring, several males, some carrying the presumed *rn<sup>2</sup>/al<sup>2</sup> Cy cn<sup>2</sup> L<sup>4</sup> sp<sup>2</sup>* genotype, were mated to *S Sp rn*, *T23/dptxI Cy pr B1 cn<sup>2</sup> L<sup>4</sup> sp<sup>2</sup>* females. The resulting *rn<sup>2</sup>/dptxI Cy pr B1 cn<sup>2</sup> L<sup>4</sup> sp<sup>2</sup>* offspring were used to establish a stock. However, several *Cy B1 L rn* flies resulted from the cross, indicating independent assortment of the *rn<sup>2</sup>* from the second chromosome.

An attempt was then made to balance *rn<sup>2</sup>* with the third-chromosome balancer *Me*, *Ins ri Sb<sup>1</sup>*, and a stock was successfully established. Crossover tests using *R* (Roughened) and *Ly* (Lyra) showed *rn<sup>2</sup>* to be located in the right arm of chromosome 3, in the neighborhood of *Sb*. From data obtained using *W* (Wrinkled) and *Sb*, *rn<sup>2</sup>* was found to lie about one-fifth of the distance between these markers, or about  $47.7 \pm 1.1$  using the standard distance of 46.0 for *W*. This was based on a total of 51 crossovers in this region, 10 of which were between *W* and *rn<sup>2</sup>*.

It is thus evident that *rn*, T23 (or the original rotund) is a reciprocal translocation with breaks of both major autosomes very near their centromeres. Probably both breaks are slightly to the right of the centromere; if so, the left arm of II is joined to the right arm of III and vice versa.

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Carson, H. L. A female-producing strain of *D. borealis* Patterson.

Fourteen wild *virilis*-group females caught at Selkirk, Manitoba, in July 1952 were separated from males at collection and were allowed to exhaust the sperm which they had received in nature. Pair matings were then made with wild males captured at the same time, and strains were developed from these. All were proved to be *D. borealis* by spermatheca examination and by crossing *F<sub>1</sub>* individuals to a known strain of *D. borealis* (Univ. Texas Lab. 2077.4, Itasca State Park, Minnesota). The strain designated as Sel-12 produced an *F<sub>1</sub>* from the wild pair consisting of 34 females and no males. The small number of offspring counted is due to the fact that only one tube of larvae was saved from this cross. *F<sub>1</sub>* Sel-12 females were crossed to *D. borealis* males from three other strains--Sel-5, Sel-7, and the unrelated 2077.4. From each of these crosses only females were produced: these

numbered 99, 191, and 286, respectively. The female-producing strain was maintained thereafter for 8 generations by crossing 10-20 females en masse with 10-20 from Sel-5. As each generation emerged, at least 50 females were counted before the backcross to Sel-5 was made. Over these 8 generations and up to the time that the strain was accidentally lost in June 1953, 1327 females and no males were observed by actual count. The females were vigorous and produced very large quantities of offspring throughout the experiment. Brain smears of these females revealed no chromosome abnormalities; they showed the typical diploid montana-complex configuration of four pairs of rods and one pair of J's; salivary-gland-chromosome smears showed three inversions, two in chromosome 3 and one in chromosome 5. Individual females were found which were homozygous both for and against each of these inversions.

Certain genetic explanations of this female production may be eliminated. The source of the male has no effect on sex ratio, and as the strains from which males were taken were wholly normal in this respect, "sex ratio" of the male-influenced type, as in D. pseudoobscura, is thus precluded. The females will not reproduce unless mated, so that the possibility of thelytokous parthenogenesis cannot explain the data. By using the naturally occurring inversions as markers, it is possible to prove that, at least for chromosomes 5 and 3, the inheritance of the females in all-female sibships is biparental. This eliminates the possibility of diploid parthenogenesis set in motion by sperm entrance without syngamy (gynogenesis). All existing details of this case suggest similarity to the case described by Magni for D. bifasciata (Proc. 9th Intern. Congr. Genet., II: 1213, 1954), in which an extra-chromosomal cytoplasmic factor, transmitted only by females, is lethal to male zygotes.

Castiglioni, M. C. Phenogenetics of pseudotumor in D. melanogaster.

In some stocks under investigation the production of benign melanotic masses seems to be conditioned by a series of processes controlled by different and independent genetical factors. The production of melanotic masses depends on: (1) The presence of a large number of a special kind of large migrant cells (hemocytes?), originating in the lymph gland, which have the tendency to clump and to produce melanin, (2) Disintegration of one portion of the lymph gland. Melanization occurs only when the big migrant cells are released in large quantity in the hemolymph, but their presence does not necessarily cause melanization. The number of these cells probably depends on their frequency in the lymph gland, although direct evidence is lacking. The total number of cells, and the frequency of big migrant cells in particular, has been determined for 11 stocks, in smears of body fluid (hemolymph) stained with May Grünwald-Giemsa. The histology of the lymph gland has been studied with microtome sections, as well as in toto.

Chung, Y. J., Paik, Y. K., Kim, D. U., and Kim, K. W. Further information regarding Drosophilid species in Korea.

We made a first report on Drosophila species found in Korea in DIS-29. As we had many other Drosophilid species in our field collections from May to October, 1956, at Kwangju, Mt. Chiri, and Quelpart Island, the following species can be added:

Only a few species of Drosophila in Korea were reported by Kikkawa and Peng, and by Nakayama, in 1936 and 1940 respectively. Since then no one has reported Drosophila species in Korea.

D. lutea, D. immigrans, D. histrio, D. bizonata, D. transversa-complex, D. nigromaculata, D. cheda, D. sternopleuralis, D. buskii, D. coracina, D. puncticeps, D. albalalis, D. histrionides, D. quadrivittata, D. (Hirtodrosophila) sp, Amiota alloguttata, Amiota (Phortica) sp, Scaptomyza disticha, Scaptomyza graminum, Leucophenga maculata, Leucophenga sp, Microdrosophila congener, Microdrosophila sp-1, Microdrosophila sp-2.

Edington, C. W. The production of dominant lethals by gamma rays, 1-Mev neutrons, and 14-Mev neutrons.

also a great difference in the ion density produced in tissue by fast neutrons of different effective energies, a comparison was made of the effectiveness of fast neutrons of 1 Mev and 14 Mev and of  $\text{Co}^{60}$  gamma rays in the production of dominant lethals in *Drosophila*. Oregon-R males, 2-4 days old, were exposed to one of the three radiations at several doses and mated to virgin Oregon-R females for 24 hours, and the percentage hatch of the eggs was determined. The following results were observed:

Gamma rays			14-Mev neutrons			1-Mev neutrons		
Dose ( $r \times 10^3$ )	Eggs laid	% hatch	Dose ( $rep \times 10^3$ )	Eggs laid	% hatch	Dose (rep)	Eggs laid	% hatch
0	1294	98.3	0	2632	97.8	0	2933	96.9
1	1130	83.8	1	1780	64.7	463	2719	68.1
2	1017	70.4	2	2389	40.0	1083	3053	37.1
3	987	51.6	3	837	22.0	2154	3037	14.9
4	1093	37.1	4	1640	11.2	2922	3830	6.8
5	1120	27.8						
6	768	23.7						

It is obvious from these data that both types of neutrons are more effective than gamma rays in producing dominant lethals and that 1-Mev neutrons are more effective than 14-Mev neutrons. Furthermore, the shape of the hatchability curve for 1-Mev neutrons and 14-Mev neutrons is linear whereas the gamma-ray data depart significantly from linearity. These results indicate that the RBE of fast neutrons of different energies decreases with decreasing ion density and that the frequency-dose relation for neutrons of high energy is linear.

Fahmy, O. G., and Fahmy, Myrtle J. Differential response of specific gene loci to mutagens in D. melanogaster.

mutations. Hundreds of these visibles have been induced on the X chromosome alone by the chemical agents; this does not seem to have been encountered in the radiation mutagenesis work. In order to determine the degree of response of these chemically mutated loci to radiation, we analyzed the mutability of 17 specific "new" visibles under the effect of X-rays. The selected loci were spread along the whole length of the X chromosome, and were most easily recognizable, and each had occurred more than once (mostly 3-5 times) in the chemical mutagenesis work. All mutants were fully viable and fertile in the homo-

We have previously reported (DIS-29; Fahmy and Fahmy, 1956a) that selective mutagenicity of the alkylating compounds as compared to radiation is manifested in the differential yield of "visible"

zygous condition. It was further possible to combine these mutants in sets of 2-4 loci per stock without impairing either the fertility or the viability. For the sake of comparison we also tested the mutability of 7 X-chromosome loci which are known to be affected (to various degrees) by radiation: scute (sc), cut (ct), vermilion (v), wavy (wy), garnet (g), forked (f), and carnation (car).

Females homozygous for the "new" visibles were mated to irradiated males carrying the normal allelomorphs, and the  $F_1$  daughters were scored for the genes tested. About 30,000 to 80,000 daughters receiving the marked and tested X chromosomes were scored per locus, and the X-ray dose ranged from 2,500 to 4,250 r. The  $F_1$  daughters showing the character tested for were further analyzed to determine whether the treated chromosome carried (1) a visible allelomorphic to the marker, (2) a visible together with a lethal somewhere else on the chromosome, or (3) a deficiency or a deletion (in itself a lethal) covering the locus of the marker.

Of the 17 chemically mutated visibles only one was affected intragenically by radiation (at a rate of  $8.4 \times 10^{-8}$  per r) and also eliminated within deletions (at a rate of  $1.05 \times 10^{-8}$  per r). Eight other visibles were eliminated within deletions--6 in the euchromatin at a rate of  $1-2 \times 10^{-8}$  per locus per r, and 2 near the heterochromatin, at a much higher rate,  $6-8 \times 10^{-8}$  per locus per r--but none of these 8 were mutated intragenically. The remaining 8 visibles were stable to radiation in the size samples utilized. That this size sample is reasonably adequate is shown by the fact that it was sufficient for the induction of intragenic mutations, as well as deletions for the tested known loci, and at roughly the same rate as ascertained by other radiation geneticists.

Details of this work will be soon published elsewhere, but the above brief note is sufficient to indicate that the results on mutability at specific loci add further support to our claim that the mutation process is not random but selective, and is dependent on the nature of the mutagen.

Fahmy, O. G., and Fahmy,  
Myrtle J. The mutagenic  
action of alkyl sulphonates.

In a recent paper (Fahmy and Fahmy, 1956b) it has been shown that a particular sulphonate (2-chloroethyl methanesulphonate:  $\text{ClCH}_2\text{CH}_2\text{OSO}_2\text{CH}_3$ ) exerts a

unique mutagenic effect on the male germ line of *Drosophila*. This compound proved to be practically ineffective on mature sperm and late spermatids, but extremely active on the early germ mother cells, particularly the spermatogonia. Furthermore, in these latter cells, the sulphonate induced practically as many sex-linked recessive visibles as lethals.

An attempt was made to determine whether these mutagenic properties are characteristic of this compound only or are shared by related sulphonates. For this purpose we tested the mutagenic action of the unsubstituted sulphonate (ethyl methanesulphonate:  $\text{CH}_3\text{CH}_2\text{OSO}_2\text{CH}_3$ ). This compound was administered by injection into adult males of the same age and average size as those used with the chloro derivative, and the progenies of these males were also fractionated in exactly the same manner, that is, in 3-day broods. The yield of sex-linked recessive lethals and visibles in the separate broods was determined by the Muller-5 technique. The concentration injected was  $1.6 \times 10^{-2}$  M and the volume received per male was 0.25  $\mu\text{l}$  of solution, making the absolute dose  $4.0 \times 10^{-9}$  mole per male. The X-linked recessive mutations

induced by the above concentration in the successive broods are tabulated below.

Brood	Chromosomes tested	Visibles	Lethals
		No. %	No. %
I	458	23 5.0	84 18.3
II	350	13 3.7	51 14.6
III	301	17 5.6	47 15.6
IV	383	9 2.3	46 12.0
V	316	- -	1 0.3
VI	345	1 0.3	2 0.6
VII	328	- -	3 0.9
Total	2481	63 2.5	234 9.4
Ratio visibles/lethals		0.27	

It is clear that the mutagenic action of the ethyl methanesulphonate is completely different from that of the chloro derivative. The unsubstituted compound is most active on the mature sperm and spermatids and practically ineffective on the early germ cells. Furthermore the efficiency of ethyl methanesulphonate in mutating morphogenesis loci (visibles) is much lower than that of chloroethyl methanesulphonate, the over-all ratio of visibles to lethals for the two compounds being 0.27 and 0.47 respectively.

The drastic alteration, almost complete reversion, of the mode of mutagenic action of ethyl methanesulphonate by the substitution of a Cl atom for an H atom of the ethyl group is yet another example of the intricate biochemical nature of mutagenesis and its dependence on the agent.

Farnsworth, M. W. Localization of alkaline phosphatase in lethal embryos and larvae of Minute(4) and Minute(1)o.

kidney epithelium, as well as in other tissues, is well known. Moog (Biol. Bull. 86: 51-80, 1944) and Yao (Quart. J. Micr. Sci. 91: 79-108, 1950) have associated alkaline phosphatase with the onset of histo-differentiation in chick and *Drosophila* embryos, respectively. Yao reported that this enzyme suddenly appears shortly after germ-band contraction (9 hours), and considered its point of origin to be the "differentiation center" of the *Drosophila* embryo. Studies of embryos homozygous for eight different Minutes have shown that the anomalies of all stocks originate around 10 to 12 hours of development, a period when alkaline phosphatase activity is presumably becoming widespread. Furthermore, all homozygous Minutes so far investigated are characterized by abnormalities of the midgut and slowness of yolk withdrawal (Farnsworth, Genetics, in press). In view of these findings, both an egg and a larval lethal were tested for the presence and localization of alkaline phosphatase.

Ten to sixteen-hour embryos of M(4) and M(1)o were used, as well as hatched homozygous first-instar M(1)o larvae and their control sibs. Initially, two fixatives were employed: chilled 80% ethanol and chilled 100% acetone. After sectioning at 6 to 10  $\mu$ , material fixed by both procedures was prepared by the method of Gomori (Microscopic Histochemistry, U. of Chicago Press, 1952) and by the method given by Yao (loc. cit.) and outlined

The role of phosphatases in the transfer of various substances across cell membranes has been reported by many authors. In particular, the presence of these enzymes in vertebrate intestinal and

by Danielli (J. Exp. Biol. 22: 110-117, 1946). It was found that fixation in 80% alcohol followed by the technique of Danielli gave the best and most consistent results, and this procedure was then followed throughout the rest of the study. Sodium- $\beta$ -glycerophosphate was used as substrate, and incubation at 37° C was varied from 2 to 20 hours, although 4 hours was used in most instances. Control slides were incubated without substrate. Additional controls used as a check on the incubating mixture consisted of frog skin and kidney, tissues previously tested and known to be highly active.

In general, no differences between controls and lethals were found with respect to the localization of this enzyme. In embryos, alkaline phosphatase appeared at the proper time, and its presence in salivary glands, Malpighian tubules, hypodermis, and to a much lesser extent the gut, was noted. The quantity of the enzyme, as compared with that in frog skin or kidney incubated for equal periods of time, was extremely low. Indeed, frog tissue required only a 15-minute incubation period for an intense and specific reaction, whereas Drosophila material, at the end of 4 hours, was only faintly positive. Long periods of incubation (12 or more hours) gave darker staining, but also resulted in diffusion artifacts, as judged from the presence of nonspecific nuclear adsorption.

Only first-instar larvae were studied, since M(1)<sup>o</sup> homozygotes do not grow beyond this stage. As reported by Yao and found in the present work, only a very weak reaction can be obtained in larvae of this age. Again, no specific differences between controls and lethals were observed.

It is concluded that the presence and localization of alkaline phosphatase is not a significant factor in the causes of lethality in Minute homozygotes.

(Supported by a grant from the American Cancer Society on recommendation of the Committee on Growth of the National Research Council.)

Farnsworth, M. W. Somatic mitosis in M(2)1<sup>2</sup> homozygotes.

As part of an investigation dealing with the quantitative determination of DNA, RNA, and total protein in various states of larval growth in wild-type, Minute heterozygotes, and Minute homozygotes, studies of somatic mitoses in aceto-orcein brain smears have been carried out. Homozygous M(2)1<sup>2</sup> larvae were used as material. Such larvae are approximately 1 mm in length and do not increase appreciably in size although they may live for several days. In smears prepared from approximately 50 of these individuals, the giant neuroblasts seemed fewer in number than in the wild type, although no cell counts were made. In addition, mitotic figures were exceedingly rare--only 6 were found in the material examined. Apparently, in Minute homozygotes, not only is increase in cell size greatly restricted or completely eliminated but, in addition, growth by increase in cell number is greatly reduced in tissues which normally undergo cell division in larval life.

(Supported by a grant from the American Cancer Society on recommendation of the Committee on Growth of the National Research Council.)

Frydenberg, Ove. D.  
pallidipennis from Peru.

D. pallidipennis centralis from Mexico is known to be genetically isolated from D. p. pallidipennis from southern Brazil by  $F_1$  male sterility. As to gene arrangement, the two subspecies differ only in a single inversion in chromosome D. Strains of D. pallidipennis from Peru have been testcrossed to South Brazilian strains of D. p. pallidipennis. There appeared to be no hybrid sterility whatsoever between the strains. The Peruvian strains are therefore regarded as belonging to the subspecies D. p. pallidipennis. However, the Peruvian strains have the same gene arrangement in chromosome D as has the subspecies D. p. centralis. Consequently there seems to be no direct connection between the differentiation of the gene arrangement and the development of isolating mechanisms in this case. A new inversion in chromosome A was discovered in the Peruvian strains. The results are to be published shortly.

Frydenberg, Ove. Two new species from Peru.

described. The description will appear in the *Revista Brasileira de Entomologia*.

Fung, S. T. C., and Gowen, J. W.  
 A major locus for sex determination in the third chromosome.

Different diploid and polyploid combinations of the genes +, Hr, and tra have been made for the study of their sex types. Besides the familiar normal males and females, the triploid type of Bridges, the two Hr types of our stock, and the two male types of Sturtevant, there are now three other distinguishable sex phenotypes. The diploid genotype Hr/tra had male-like but retracted genitalia and no visible claspers, sex combs of 8-9 teeth, internal genitalia largely male. The Hr/tra/tra resembled the Hr/tra male, sex combs 8-9 teeth, internal genitalia predominately male, well developed duct and accessory organs, testes elongated slightly but much smaller than normal males. The external genitalia of Hr/tra/+ flies resembled diploid Hr/+, had rudimentary claspers and sex combs of 5-6 teeth. The internal genitalia were developed, with mixture of male and female sex organs. Besides the intersex genotype, 2X + 3A, which was little affected by these genes, there were ten distinguishable sex types which were produced by the action or interaction of these genes in the diploid and triploid flies. Dosage interactions in diploids and triploids proved that + of the wild type was like the Hr and tra, a sex gene. These genes were in the third chromosome. The results show that fundamental sex characters, like other characters, sometimes may be due to substitution of major sex genes occupying particular loci.

Gersh, Eileen Sutton.  
 Salivary analysis of Y:bw<sup>+</sup>.

The piece of chromosome 2 inserted in Y extends over a little more than one numbered section of Bridges' map. Its minimum extent is from 59E1.2 to 60E3, and a few more bands may be included at each end. It is visible in salivary-gland nuclei as a loop situated at the chromo-center, and constitutes a useful salivary marker by means of which an extra Y chromosome (specifically Y:bw<sup>+</sup>) can be detected.

Glassman, Edward. Allelism between the tumor-producing loci of the *ell* tu and *bw* tu strains.

Genetic analysis indicates that the tumors of the *ell* tu stock are dependent upon a single gene located at about 88 on the second chromosome. It has been reported (Hartung, 1950, *J. Heredity* 41: 269) that

the tumor gene in the *bw* tu stock is on the same chromosome at about 84. A cross between the two stocks produced 334/435 (76%) tumorous offspring, and since both stocks show very low penetrance when heterozygous, these genes are probably allelic. The differences in linkage data are most likely a result of inaccuracies due to the incomplete penetrance of these genes.

Glassman, Edward. The occurrence of urea in *D. melanogaster*.

According to various authors, the end products of nitrogen metabolism in insects are the relatively insoluble uric acid

and allantoin. Since large amounts of uric acid and some allantoin were detected on paper chromatograms of *D. melanogaster* extracts, it was unexpected to find that a compound accumulated by a sable (body color) strain exhibited *R<sub>f</sub>* values similar to urea in 5 solvents. The fact that the compound forms a yellow spot with Ehrlich's reagent (p-dimethylaminobenzaldehyde, applied as a 2% solution in 5% HCl), and is destroyed by urease, indicates further that it is urea. Many stocks show traces of this compound, but it is most evident in the sable strain. Genetic analysis is in progress. The source of the urea is not known, but since large amounts of what appears to be allantoin are also present in these flies, as well as in their excreta, a relation may exist between them.

Henke, H., Hühne, G., and Kunkel, H. A. Recessive sex-linked lethals in successive broods of *D. melanogaster* after N oxide mustard treatment.

It has been evidenced by a number of investigations that during spermiogenesis in *Drosophila* the germ cells pass through some periods of different sensitivity to the effects of chemical mutagens.

Furthermore, different kinds of mutagenic action has been found among these agents. As indicated by Auerbach's results with *Drosophila*, mustard gas produces the highest mutagenic effect in a period of sperm development intermediate between early spermatogonia and mature spermatozoa. In our investigations a new derivate of the nitrogen-mustards, bis-( $\beta$ -chloroethyl)-methylamineoxidehydrochloride, was tested for frequency of induction of mutations in successive stages of sperm development in *D. melanogaster*.

An aqueous solution (1.0%) of the nitrogen oxide mustard was injected intraabdominally one day before copulation. The treated males were given fresh females every three days, and the rates of recessive sex-linked lethals were determined by the Muller-5 technique in five successive broods. As is demonstrated in the table, no mutagenic effect was obtained in very early periods of sperm development. The rates of mutation found in the following stages of spermiogenesis and in mature sperm show particular differences. Thus the sensitivity to the mutagenic effect of nitrogen oxide mustard increases to a maximum in the third brood. The mutation rate here is twice as high as in the second or the fourth brood, and exceeds the frequency of mutations in mature sperm. A similar type of mutagenic action has been established by Auerbach's investigations with mustard gas. In relation to the correspondent stages of spermiogenesis determined by Auerbach, the maximum of mutation sensitivity to nitrogen oxide mustard shown in the table

occurs during or soon after meiosis. This kind of action differs somewhat from the mutagenic effect of X-rays.

Brood	Copulation intervals after treatment (days)	No. of X chromosomes tested	Induced lethals No.	Induced lethals %
I	2-4	2678	71	2.65
II	5-7	2149	35	1.63
III	8-10	2178	74	3.40
IV	11-13	971	16	1.65
V	14-16	1537	4	0.26

Herskowitz, Irwin H. Studies on the nature of recessive lethal mutations induced in oocytes by X-rays.

It was reported (DIS-29: 125, 1955) that a concentrated treatment of oocytes with about 3264 r produced significantly more sex-linked recessive lethals than did this dose delivered in a protracted manner. A similar result was obtained, although the difference was not significant, in a subsequent experiment using 4000 r, in which  $8.1 \pm 1.6\%$  lethals (25/308) were obtained with the concentrated treatment and  $5.9 \pm 0.8\%$  lethals (53/905) with the protracted. Even though, in both experiments, the  $F_1$  tested for lethals came from eggs oviposited within 4 days after irradiation, the results might have been due to an intensity effect on oviposition rate and not on mutation rate. For there is an intensity effect on oviposition rate, proved at about 2000 r with dehydrated females (Anat. Rec. 125: 639, 1956); and since the rate of induced recessive lethal mutations is known to decline in successive eggs laid, the smaller number of eggs laid after intense treatments might contain a significantly greater frequency of such mutations than the larger number of eggs laid in the same interval of time after diluter treatments.

Since no appreciable effect of intensity on oviposition rate has been found when normal (hydrated) females are given an X-ray dose of 2500 r or less, the present experiments were performed using a dose of 2300 r.

The females employed were free of recessive sex-linked lethals arising in a previous generation, and were of two types: "rod/rod," homozygous for a wild-type X chromosome; and "rod/ring," having one identical wild-type chromosome and one ring X ( $Xc2\ y\ B$ ). The "rod/rod" females were given the X-ray dose either intensely (I) in 94 seconds, or protractedly (D) over a period of 5 hours 25 minutes, or were given no dose (C); and the "rod/ring" females were given either the intense treatment (I) or no irradiation (C).

Among eggs laid the first 4 days after treatment, sex-linked recessive lethal mutation percentages for rod/rod females were: for C, 0.125% (1/799); for I,  $4.73 \pm 0.60\%$  (60/1268); and for D,  $2.89 \pm 0.41\%$  (47/1625). In the rod/ring females the identical rod-X gave mutation rates of C = 0.0% (0/377) and I =  $2.73 \pm 0.65\%$  (17/623), and the ring-X 0.0% (0/347) and  $2.98 \pm 0.69\%$  (18/605) for C and I, respectively. These values show a significantly higher mutation rate for the rod chromosome irradiated intensely (I) than for the same rod chromosome irradiated protractedly (D) when it had a rod X as its homolog ( $P = .015$ ), or irradiated intensely (I) when it had a ring X for its homolog ( $P = .05$ ).

The average number of eggs laid per fertile female in the first 4 days after irradiation was for rod/rod females 46, 41, 42, for I, D, and C respectively, and for rod/ring females 55 and 50 for I and C, respectively. Thus the oviposition rate of irradiated flies was not less than in the unirradiated controls, and it is unlikely that the approximately 20% greater number of eggs laid after intense treatment by rod/ring females than by rod/rod could account for the significantly higher mutation rate of the rod X in the latter type of female as compared with the former.

The intensity effect on lethals demonstrates that a considerable proportion of such mutations induced in oöcytes are multi-hit events. Since it is known that broken ends produced by X-rays in oöcyte chromosomes can join soon after their production, it is suggested that the intensity-dependent lethals are connected in their origin with multi-break exchanges. Such exchanges could include small deficiencies and duplications acting as recessive lethals, produced by "pseudo crossing over"--intra-tetrad exchange between nearby but nonhomologous loci. Supporting this view is the lower lethal rate for a rod when its homolog is a ring rather than a rod, for pseudo crossing over in the former case would much more often form a dicentric, which would not be included in the haploid egg.

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Herskowitz, Irwin H. The effect of dehydration upon the frequency of X-ray-induced crossover-like exchanges in oöcytes and oögonia.

whether X-ray-induced exchanges of crossover-like nature likewise were sensitive to prior dehydration of the mothers, was performed in part to test whether the dehydration-increased egg mortality could have been the result of an effect on the genetic material.

Virgins ( $y\cdot Dp\ sc^{V1} y^+/y^2 v\ f\ car$ ) were dehydrated (D) or not (W) and irradiated (I) or not (C) with 2940 r delivered in 2 minutes 5 seconds (furnishing four series, DI, DC, WI, WC) and then mated en masse to equal numbers of  $y^2 v\ f\ car/Y^+$  males in nylon-mesh-covered egg-laying cylinders. The cylinders were placed on nutrient-containing Petri dishes and the dishes replaced twice daily for 20 days. Egg counts were made for all 40 of these 1/2-day periods, and for a number of these periods adults were obtained from the eggs and scored for phenotype.

Some of the data are summarized in the table. For eggs oviposited within 4 1/2 days after treatment (periods 1-9), which were at the time of irradiation late oöcytes presumably past the stage in which spontaneous crossing over is thought to take place, there were in the car-centromere region significantly more exchanges induced by DI than by WI treatments. This was true also for the egg-laying periods 14-16, but not for periods 39-40. Since dehydration and a dose of 2940 r both inhibit egg laying, the data for different treatments should be compared also on the basis of approximately equal successive groups of eggs laid. Since in the first 8-day period of egg laying the percentages of induced exchanges for both DI and WI were rising, examination of the data in this way made the difference already noted

A higher egg mortality, among eggs laid over approximately the first 8 days after irradiation, had been found when dehydrated females rather than normally hydrated ones were X-rayed. The present experiment, to determine

in this period even greater, whereas there was still no difference in the 39-40 periods. Thus dehydration increases the frequency of X-ray-induced cross-over-like exchanges as it does egg mortality, the dehydration effect extending over a similar period of egg laying in both cases.

1/2-Day ovipo- sition periods	% DI- (A)				% WI- (B)			A-B
	DI	DC	% DC	WI	WC	% WC		
1-9	No. F <sub>1</sub>	400	1969	2531	2116			
	% car- centromere exchanges	8.25 ±1.34	2.69 ±0.36	5.56 ±1.42	5.13 ±0.43	2.69 ±0.35	2.44 ±0.56	3.12±1.53 (P<.05)
14-16	No. F <sub>1</sub>	1846	631	2532	668			
	% car- centromere exchanges	12.1 ±0.76	3.3 ±0.51	8.8 ±1.04	7.0 ±0.51	1.6 ±0.49	5.4 ±0.69	3.4 ±1.25 (P<.01)
39-40	No. F <sub>1</sub>	2974	835	4629	569			
	% car- centromere exchanges	6.4 ±0.45	2.9 ±0.58	3.5 ±0.73	6.0 ±0.35	3.0 ±0.72	3.0 ±0.80	0.5 ±1.1

These results permit one to propose that the dehydration effect on the egg mortality produced by radiation has a genetic basis. Since X-ray-induced exchanges of the type scored here were earlier shown to be multi-hit events, and some of these at least are multi-break events, it is suggested further that dehydration affects rearrangement frequency. A possible way of doing this would be by shrinking the nucleus and bringing independently produced broken ends closer to each other, proximity of such ends favoring interchange. The results establish that the exchanges in eggs laid 7-8 days after irradiation, considered to be X-ray-induced crossovers, are also dehydration dependent, as well as, as previously shown, intensity dependent.

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Herskowitz, I. H., and Myers, Terry. Mortality induced by an X-ray dose given to sperm intensely and to oöcytes intensely and protractedly.

After it was found that egg mortality after X-radiation of oöcytes was in large measure intensity dependent (Abrahamson and Herskowitz), and that some genetic events (such as half-translocations and pseudo-crossovers) responsible for this are much more frequent after irradiation of oöcytes than of sperm (Herskowitz and Schalet; Muller and Herskowitz), it became desirable to compare the effectiveness, in producing mortality in the egg, larval, and pupal stages, of a concentrated X-ray dose administered to sperm with that of the same dose applied to oöcytes in both a concentrated and a protracted manner.

Accordingly, virgin wild-type males and females, free of sex-linked recessive lethal or sublethal mutations arising in a previous generation, were collected and stored for 2-4 days, after which they were both divided at random into four groups: "C" in which neither sex was irradiated, "I<sub>0</sub>" in

which only males were irradiated with 2300 r delivered intensely, "I♀" in which only females were given 2300 r delivered intensely, and "D♀" in which only females were treated with 2300 r delivered in a protracted manner. The concentrated dose was delivered at 940 r per minute in 2 minutes 28 seconds; the protracted at 33 r per minute in 7 irradiations, each 10 minutes long, with 30-minute nontreatment intervals between successive irradiations. The X-ray machine was run at a peak of 200 kv and 20 ma, and 1 mm Al was used to filter the rays. The single intense irradiation was given simultaneously to males and females midway in the course of the protracted irradiation treatment. All flies were motile and well aerated during the irradiation.

Beginning about one-half hour after the irradiations were completed, the flies were etherized and placed either in single pairs in the individual compartments of the egg-laying chambers (described in detail by Abrahamson and Herskowitz), to determine egg mortality; or large and equal numbers of males and females were placed in uncompartmented egg-laying chambers to provide additional larvae for the larval and pupal mortality studies. The egg-laying chambers were placed on nutrient-containing Petri dishes, which were replaced twice daily. The males were left with females for the entire 4 days after irradiation that eggs were collected. The results are summarized in the accompanying table.

Oviposited on days	Eggs				Larvae				Pupae			
	1/2-2		3-4		3-4		3-4		3-4		3-4	
	No.	% mortality	No.	% mortality	No.	% mortality	No.	% mortality	No.	% mortality	No.	% mortality
C	1111	2.5	1845	1.8	800	15.2	638	27				
I♂	2095	46.0	4171	35.4	800	15.0	643	25				
I♀	1143	37.3	3557	31.1	800	23.6	575	31				
D♀	1217	32.4	3489	14.9	600	15.7	591	24				

The already-mentioned intensity effect on egg mortality when oöcytes are treated is found here also. Although the egg mortality for I♂ is in general higher than for I♀, it varies according to period, that at 1/2-2 days for I♀ being higher than that at 3-4 days for I♂. The rate in the I♂ is lower in the later (3-4 day) period than the earlier (1/2-2 day) one, probably because by that time the highly mutable sperm delivered in the first copulation were diluted by less mutable sperm of a subsequent copulation. While there does not appear to be any increase in mortality in the larval and pupal stages over the control rate after the I♂ or D♀ treatments, this does seem to be the case after the I♀ treatment. This suggests that a concentrated dose of 2300 r to oöcytes produces multi-hit events (probably genetic), which kill in later developmental stages and which are less frequent when this dose is delivered to oöcytes protractedly or to sperm intensely. It is thought probable on the basis of other work (Herskowitz) that these events are pseudo-crossovers.

(This work has been supported by a grant to Dr. H. J. Muller and associates from the American Cancer Society.)

Hexter, W. M. Pseudoallelism at the  $g$  locus.

Tests for crossing over between  $g^{53d}$  and  $g^2$ -Caltech in attached-X chromosomes appropriately marked on both sides of garnet resulted in 7 wild-type females in 78,000 females tested. Four of the 7 wild-type females yielded progeny, which indicated that each wild-type female was associated with a recombination of the markers, strongly suggesting that they resulted from a crossover between pseudoallellic loci. The other 3 wild-type females gave no offspring. Three of the four crossovers were reciprocal and one was nonreciprocal. The presumed double garnet ( $g^{53d} g^2$ ) chromosome has been detached, and tests to verify conclusively that both garnets are present are now in progress.

Hinton, Claude W., and Schmidt, Jean A. A variegated  $sc^8.Y$  chromosome.

A compound  $w^{vc}+sc^8.Y$  female which received 1000 r X-rays produced among her  $y w^{spl} sn^3/sc^8.Y$  sons one which was strongly yellow variegated. Tests of the  $sc^8.Y$  chromosome (called  $sc^{8V}.Y$ ) carried by this male indicate the variegation is a typical V-type position effect. The variegation is partially suppressed in  $y^{GXY}L$ ,  $sc^8$ ,  $dl-49$ ,  $y v f$  car/ $sc^{8V}.Y$  males as compared with  $dl-49$ ,  $y v f$  car/ $sc^{8V}.Y$  males; and males of the latter genotype reared at  $18^\circ C$  exhibit more variegation than those reared at  $26^\circ C$ . The variegation is manifested in all males carrying  $sc^{8V}.Y$ , and from 10 to 15 per cent of the bristles on the dorsal thorax of such males are yellow.

Hinton, Taylor, and Dagg, Martha Kushida. The lethals associated with twenty-two related chromosomal arrangements.

analyses were made (Hinton, 1950). They were balanced with Cy. The lethals associated with further chromosomal lateration. Of these stocks, the following were used in the present study: IIA, IIP, IIV, IIBI, IICQ, IICR, IIDC, IIDH, and IIDJ. These ten stocks will be referred to as the reversion series.

Phenotypically, In(2LR)40d is identified by an abnormal eye, and is lethal in the homozygous condition. Further irradiation of the inversion was carried out, and twenty-four reversions of the eye phenotype were isolated and cytological

It was disclosed that the reversions were

reverted completely to normal. The combination IIDD/In(2LR)40d was lethal.

Samples of In(2LR)40d and In(2LR)IIDD were further irradiated, and offspring which genetically manifested translocations involving the second and third chromosomes were selected. These were balanced with Cy; D.

The descriptions of most of these genetically selected translocations have appeared in previous issues of DIS. The ones used in the present study are: T(2:3)Hin 102, T(2:3)Hin 105, T(2:3)Hin 106, T(2:3)Hin 111, T(2:3)Hin 114, T(2:3)Hin 119, T(2:3)Hin 120, and T(2:3)Hin 121. These eight stocks will be referred to as the translocation series.

This varied collection of chromosomal arrangements in the two series, all derived from the same arrangement, has been tested in all possible combinations two by two in order to determine whether the lethality associated with the original one is allelic to the others (Dagg, 1955). Since Cy is used to

balance the second chromosome in all cases, the presence of non-Cy in the offspring of a cross would indicate that allelic lethals did not exist between the test stocks. All crosses were repeated at least twice.

Six of the stocks in the translocation series (T(2:3)Hin 102, 106, 111, 114, 119, and 120) proved to have a lethal that was allelic to the lethal in the original inversions, In(2LR)40d and In(2LR)IIDD, and in all of the stocks in the reversion series, except IIDC. The lethal shared in common by these seventeen stocks will arbitrarily be referred to as lethal #1.

One stock in the translocation series (T(2:3)Hin 105) had a lethal that was allelic only to a lethal in In(2LR)IIDD and not to those in any of the other stocks. This lethal has been designated #2. In(2LR)IIDD had previously been shown to contain lethal #1.

A lethal (#3) was found in T(2:3)Hin 121 not allelic to any of the other lethals in this study.

A lethal (#4) was found in one stock in the reversion series (IIDC) which was allelic only to T(2:3)Hin 119, which was also shown to contain lethal #1. The IIDC lethal had previously been found to be allelic with another stock in the reversion series, IICO (Hinton, 1950). The IICO stock was lost before the present study was begun and thus Hin 119/IICO combination could not be tested. Hinton (1950) also reported that IICO and Plum had a lethal in common (#5) but IIDC and Plum did not. Likewise Hin 119 and Plum do not have a lethal in common.

It is possible to conclude that the same region of the chromosomes is responsible for the lethality in all of the cases (rather than having to postulate five different lethals in the series) if the lethaliies are the result of interrupting the sequential action of a region of the chromosome concerned with some vital function. Two lethals will act as alleles only if between them they fail to supply all the steps in the sequence.

Horikawa, M. Tryptophan metabolism in the eye discs of D. melanogaster in tissue culture.

this issue), to investigate tryptophan metabolism in the eye discs. In comparison with culture of the eye-antennal discs alone, the culture of the eye-antennal discs together with the cephalic complexes showed more pronounced growth, differentiation, and pigmentation of the eye discs.

In the synthetic medium containing 5 mg/ml L-tryptophan, brown pigment was deposited in the eye discs of Oregon and bw after culturing for about 72 hours. In the medium containing 4 mg/ml DL-kynurenone, the pigment was deposited in the eye discs of Oregon, bw, and v after culturing for about 30 hours. The eye discs of v bw, however, deposited pigment after 55 hours. In the medium containing 2 mg/ml DL-3-hydroxykynurenone, the eye discs of Oregon, v, cn, and bw deposited pigment after culturing for 5 hours, whereas the eye discs of v bw and cn bw deposited pigment after about 15 hours. Amounts of pigments deposited in the eye discs of v bw and cn bw were less than those of Oregon, v, cn, and bw.

The eye-antennal discs and cephalic complexes from mature third-instar larvae (95 hours after hatching at 25° C) of D. melanogaster were cultured in vitro in a synthetic medium (see Technical Notes,

The fact that in the medium containing 2 mg/ml DL-3-hydroxykynurenine the eye discs of Oregon deposited brown pigment after culturing for 5 hours seems to show that all enzymes relating to the tryptophan metabolic system were present in the eye discs of the mature third-instar larvae of Oregon.

The smaller amount of pigment in the eye discs of the double recessive mutants, v bw and cn bw, seems to indicate that there may be some interaction between tryptophan metabolism and pteridine metabolism.

Hunter, Preston E. Observations on length of larval and pupal periods in D. melanogaster.

In a study conducted at the Drosophila laboratory of the University of Kansas, in which separate lines of melanogaster were selected for long and short larval periods, respectively, particular attention was paid to a correct record of larval and pupal periods. The larvae were kept individually in small medium vials and checked for pupation and emergence at regular intervals throughout a 24-hour period. In both selected and control lines the female flies always had a shorter pupal period than male flies. Average differences in length of pupal period between females and males for a representative sample of 18 generations were: short larval period line,  $5.8 \pm 0.59$  hours; control line,  $5.4 \pm 0.64$  hours; long larval period line,  $5.0 \pm 0.72$  hours. No difference in length of the larval period was found between male and female flies. In contrast to adult emergence, which occurs chiefly in the early morning hours, it was noted that in all lines studied pupation occurred uniformly throughout any 24-hour period.

Jacobs, M. E. Studies on melanism in D. melanogaster.

From a grocery garbage can at Beaufort, North Carolina were selected a light strain (wild type), a dark strain with dark trident and scutellum and slight darkening of the sclerites in general (but less dark than the mutant black), and an ebony strain with light puparia. The dark and ebony genes are semidominant alleles of the laboratory mutant, ebony. The mean larval period is: light (shortest), dark (intermediate), and ebony (longest). Colorimetric determination of tyrosinase activity of mature larvae showed: light (least, dark (intermediate), and ebony (greatest). Amino acid determinations of mature larvae by means of two-dimensional paper chromatography showed ebony to have more tyrosine and less of an unknown than light larvae. Ebony larvae that were fed methionine showed more of the unknown and less tyrosine than ordinary ebony larvae. Colorimetric determinations of tyrosine confirmed the chromatographic findings.

Kato, Mikio, and Kato, Masaru. Lipids in some mutants of D. melanogaster.

The following stocks may be divided into three groups according to color of lipochromes: group A (+); B (v, cn, se, bw); and C (w, cn-bw, v-bw). In the B group this color is pale yellow or yellowish orange, particularly dark in cn; in the A and C groups it is a watery whitish. The appearance of saponified lipochrome in "wild" is watery, and in w is whitish cream in color, but in both cn-bw and v-bw it is a faint tint of pale yellow.

On the other hand, lipochromes in these groups present marked differ-

ences from each other in refractive index, iodine value, saponification value, neutralization value, Reichert-Meissl value, Polenske value, intensity of absorption extinction (Du-Beckman spectrophotometer). Paper chromatographic examination reveals differences in fatty acids, especially in unsaturated fatty acids; lecithin and lysolecithin are found in the B group, cepharin and choline in the C group.

Khishin, Aziz F. *Drosophila*  
in Egypt.

The *Drosophila* fauna of Egypt was never investigated until April, 1956, when the writer started to collect species in an

attempt to survey distribution and to study various problems related to suspected adaptation to environment, particularly temperature. The occurrence of a number of cases sufficiently far apart, and the possibility of their being inhabited by some *Drosophila* species, necessarily inbred and isolated, should present interesting material for population and other studies.

At the present time, the work has only begun, and collections--for the sake of convenience only--have been restricted to Cairo, and a suburb called Matareya. Collections were made during April, May, and June, a dry season with temperature seldom falling below 30° C during the daytime. Traps used were one-pint milk bottles into which over-ripe bananas were mashed. These were usually set up shortly before sunset and left for about two hours before being removed. The bottles were either tied to branches or put on the ground under trees.

So far, four species of *Drosophila* have been found: *D. melanogaster*, *D. simulans*, *D. busckii*, and species of the *repleta* group. By far the most predominant species is *D. melanogaster*, closely followed by *repleta* and *simulans*. *D. busckii* seems to be very rare, as only one female was caught over a period of three months. It may also be of interest to mention that *D. simulans*, *repleta*, and *busckii* were never found indoors, whereas *D. melanogaster* was found both in and out of doors.

Khishin, Aziz F. Pupation  
habits of *Drosophila*.

Stocks of *D. melanogaster*, *simulans*, and *repleta* caught in Cairo and suburbs were raised on laboratory food containing

baker's yeast, molasses, flour, and agar. It was noticed that in all cultures, in vials or bottles, larvae about to pupate did not crawl up the walls as usual, but instead pupated on the surface of the food. This was observed again in the second generation. However, beginning with the third generation some of the larvae started to crawl up and pupate on the walls in the usual way. Now, all the introduced *Drosophila* behave in exactly the same way as laboratory ones.

Kikkawa, H. Metal analyses of mutants of the *w* series in *D. melanogaster*.

As shown in a previous issue (DIS-29, p. 130, 1955), mutants belonging to the *w* series are divided into three or four main groups or types from the view point

of metal absorption.

- (1) Ni group ..... w
- (2) Cu group ..... w<sup>e</sup>, w<sup>e2</sup>, w<sup>t</sup>, w<sup>bf2</sup> w<sup>co</sup>
- (3) Fe group ..... w<sup>a</sup>, w<sup>h</sup>, w<sup>sat</sup>, w<sup>co1</sup>
- (4) Cu + Fe group ... w<sup>ch</sup>, w<sup>ch2</sup> (discovered by the author)

In my opinion, the first three groups (1-3), at least, may be looked upon as different genes, and furthermore mutants within one group having the same metal pattern may be looked upon as multiple alleles.

King, R. C., and Rudden, H. J.  
Studies on hybrids between  
"tumorous" strains of *D. melanogaster*.

Hybrids between the pseudotumor strains  $tu^W$  (Wilson et al., Growth 19) and  $tu^{531}$  (King, DIS-29) were studied. In the case of  $tu^W$ , pseudotumors result from encapsulation of the caudal fat masses by lamellocytes (Rizki, Anat. Rec. 125). Tumors in the  $tu^{531}$  stock are associated with an X-chromosomal mutant; whereas the factors responsible for pseudotumor expression in the case of  $tu^W$  reside in chromosome II. The most important results of the study are presented below.

		Genotype		
	<u>tu</u> incidence	<u>I</u>	<u>II</u>	<u>III</u>
1	100%	$tu^W$	$tu^W$	$tu^W$
2	50%	$tu^W$	$tu^W$	$So/D$
3	15%	$tu^{531}$	$tu^W$	$So/D$
4	5%	$tu^{531}$	$Cy/Pm$	$tu^W$
5	5%	$tu^{531}$	$tu^{531}$	$tu^{531}$
6	5%	$tu^{531}$	$Cy/Pm$	$tu^{531}$

Comparison of rows 1 and 2 shows that chromosome III of the  $tu^W$  strain enhances tu incidence in that strain. However,  $tu^W$  III does not enhance tu incidence when substituted in the  $tu^{531}$  genome (see rows 4, 5, and 6). Furthermore, the X chromosome of  $tu^{531}$ , which is responsible for tu incidence in this stock, reduces tu incidence when substituted in the  $tu^W$  genome (compare rows 2 and 3). Many pseudotumor strains exist, and the tendency has been to assume that information obtained for one strain applies to all strains. This work shows that such generalizations are hazardous. Blackened cell aggregations will probably be shown to be a generalized response to various stimuli. Factors which enhance this response in one strain may have no effect or suppress the response in other strains.

Komai, Taku, Yamada, Yukio,  
Hiraiumi, Yuichiro, and  
Kitagawa, Osamu. Effect of  
selection after X-ray  
irradiation.

Selection was conducted for larger and smaller numbers of chaetae on the fourth and fifth abdominal plates of *D. melanogaster*, starting with a cross of Oregon-R and Samarkand stocks. The flies of each successive generation were irradiated with 1500 r X-rays. The H (high chaeta number) and L (low chaeta number) lines were classified in four lots according to whether (1) both sexes, (2) only females, (3) only males, or (4) neither sex was treated. The selection intensity was 30% up to the fourth generation, and 20% in the fifth and sixth generations. Variance analyses revealed significant differences in chaeta number between H and L. Also, the lots among H lines in which only females had been irradiated showed significantly higher chaeta numbers than the lots in which only males had been irradiated. This seems to indicate that the apparent effect of X-rays on the induction of new mutations controlling chaeta number is at least partly (or perhaps mostly) due to the release of already existing genes by the X-rays through enhancement of recombination. This work will be continued by Kitagawa in the Genetics Laboratory of Tokyo Metropolitan University.

Selection was conducted for larger and smaller numbers of chaetae on the fourth and fifth abdominal plates of *D. melanogaster*, starting with a cross of Oregon-R and Samarkand stocks. The flies of each successive generation were irradiated with

Kuroda, Y., and Tamura, S.  
Effects of  $Cu^{++}$  on the melanotic growth of tumors in D. melanogaster in tissue culture.

(see Technical Notes, this issue) involving  $CuSO_4 \cdot 5H_2O$  in concentrations of 1.0 mM, 2.5 mM, and 5.0 mM.

Melanotic tumors in the hindgut of mature third-instar larvae (95 hours after hatching at 25° C) of v tu and st tu strains of D. melanogaster were cultured to investigate the effects of  $Cu^{++}$  on melanotic growth in a synthetic medium

Conc. of $Cu^{++}$ added	No. of cultures	Melanotic growth of tumors					
		Excellent		Progressive		Slight	
		No.	%	No.	%	No.	%
1.0 mM	50	6	12	25	50	19	38
2.5 mM	50	13	26	35	70	2	4
5.0 mM	50	22	44	20	40	8	16
0	50	8	16	37	74	5	10

As shown in the table, melanotic growth was inhibited markedly when 1.0 mM  $Cu^{++}$  was added to the synthetic medium. The addition of 2.5 mM  $Cu^{++}$  to the synthetic medium resulted in no significant difference from culturing without  $Cu^{++}$ . The effect of 5.0 mM in the synthetic medium was to accelerate the melanotic growth pronouncedly. In the basis of these findings it is assumed that a substance inhibiting the phenol oxidase system is present, and this inhibitory substance seems to be enhanced by 1.0 mM  $Cu^{++}$ .

Kuroda, Y., and Tamura, S.  
Effects of DDC (diethyldithiocarbamate) on the melanotic growth of tumors in D. melanogaster in tissue culture.

DDC is known to inhibit melanin formation by chelating some metals. Melanotic tumors in the hindgut of mature third-instar larvae were cultured in vitro, by the procedures described in the preceding note, to investigate the effect of DDC upon the melanotic growth of tumors. The results are shown in the table.

Conc. of DDC added	No. of cultures	Melanotic growth of tumors					
		Excellent		Progressive		Slight	
		No.	%	No.	%	No.	%
1.0 mM	50	15	30	18	36	17	34
2.5 mM	50	5	10	24	48	21	42
5.0 mM	50	0	0	2	4	48	96
0	50	8	16	37	74	5	10

It was observed that the higher the concentration of DDC in the synthetic medium was, the more markedly the melanotic growth of tumors was inhibited. The addition of 5.0 mM DDC to the synthetic medium inhibited the melanotic growth of tumors almost completely.

Kuroda, Y., and Tamura, S.  
Effects of  $Fe^{+++}$  on the melanotic growth of tumors in D. melanogaster in tissue culture.

Melanotic tumors in the hindgut of mature third-instar larvae of v tu and st tu strains of D. melanogaster were cultured in synthetic medium involving

$\text{Fe}(\text{III})_2(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$  in concentrations of 1.0 mM, 2.5 mM, and 5.0 mM. The results are shown in the table.

Conc. of $\text{Fe}^{+++}$ added	No. of cultures	Melanotic growth of tumors					
		Excellent		Progressive		Slight	
		No.	%	No.	%	No.	%
1.0 mM	50	15	30	24	48	11	22
2.5 mM	50	17	34	19	38	14	28
5.0 mM	50	22	44	19	38	9	18
0	50	8	16	37	74	5	10

When 1.0 mM  $\text{Fe}^{+++}$  was added to the synthetic medium, it was observed that progressive melanotic growth of tumors was decreased, and excellent and slight growth were increased. The addition of 2.5 mM  $\text{Fe}^{+++}$  to the synthetic medium had similar effects on melanotic growth. Addition of 5.0 mM  $\text{Fe}^{+++}$  to the synthetic medium accelerated melanotic growth pronouncedly. In relation to the effects of  $\text{Cu}^{++}$  upon the melanotic growth of tumors, described in the preceding note, these results seem to indicate that transition metals play an important role in the formation of melanotic tumors.

Kuroda, Y., Tamura, S., Abe, K., and Doi, K. Relation between hereditary tumors and transition metals in *D. melanogaster*.

for comparison with those contained in the nontumorous strains v, st, and Oregon. The results are shown in the table.

Amounts of metals contained in pupae of *D. melanogaster*, the first day after pupation, were determined in two melanotic tumorous strains, v tu and st tu, and a nonmelanotic tumorous strain, tu-h,

Expt. no.	v tu		v	
	Fe	Cu	Fe	Cu
1	294	211	270	199
2	569	225	273	154
3	320	198	308	129
Mean	328	211	284	161

Expt. no.	st tu		st	
	Fe	Cu	Fe	Cu
1	282	346	140	356
2	209	386	131	340
3	201	340	163	342
Mean	231	357	145	346

(table continued on following page)

Expt. no.	tu-h		Oregon	
	Fe	Cu	Fe	Cu
1	381	242	167	159
2	391	265	152	154
3	404	301	140	185
Mean	393	269	153	166

Greater amounts of iron and copper were detected in the tumorous strains, v tu, st tu, and tu-h, as compared with the nontumorous strains, v, st, and Oregon, respectively. As a result of these facts, it is assumed that the formation of hereditary tumors in *D. melanogaster* is closely related with the transition metals.

Kurokawa, H. Sexual isolation among the three races of *D. auraria*.

The three races (A, B, C) of *D. auraria* are distributed sympatrically in Japan. In the laboratory, these races can be intercrossed regardless of the localities of capture, though it is not so easy as intraracial crossing. The  $F_1$  hybrids of the interracial crosses are fertile in both sexes, and are intermediate between parental races in characteristics. So far, however, no natural hybrids have ever been found among many samples from localities where two or three races occur together. Judging by this fact, gene transfer among the three seems to be precluded mainly by sexual isolation.

In experiments, using the multiple-choice technique, between intraracial strains (A-A; B-B; C-C), none showed significant sexual isolation, irrespective of the strain used. In interracial experiments, on the other hand, significant isolation was detected; the results are given in tables 1, 2, and 3. Letters in parentheses indicate the races. Stalker's "Isolation Index" (I) was used for analysis of the data; significance was tested by chi square.

Table 1. A-B

Crosses		I
♀	♂	
G(A), S(B)	x G(A)	0.56
G(A), S(B)	x S(B)	0.77
G(A), K(B)	x G(A)	0.79
G(A), K(B)	x K(B)	1.00

Table 2. A-C

Crosses		I
♀	♂	
H(A), U(C)	x H(A)	0.49
H(A), U(C)	x U(C)	0.99
H(A), S(C)	x H(A)	0.57
H(A), S(C)	x S(C)	0.98
H(A), M(C)	x H(A)	0.59
H(A), M(C)	x M(C)	0.89

Table 3. B-C

Crosses		I
♀	♂	
K(B), U(C)	x K(B)	0.74
K(B), U(C)	x U(C)	0.89
K(B), S(C)	x K(B)	0.43
K(P), S(C)	x S(C)	0.66
K(B), M(C)	x K(B)	0.40
K(B), M(C)	x M(C)	0.67

Kurokawa, T. Effect of starvation on the phenotypic expression of  $vg^{NP}$  flies.

Flies of  $vg^{NP}$  stock, which normally have strap- or antlered-like wings, were raised on peptone food (partial starvation) during certain periods of larval development. (3.8 g peptone, 1 g agar, 1.7 g glucose, in 100 ml water.) When larvae were transferred to peptone food two days after oviposition and kept there until pupation, the wing phenotype of all female flies was shifted toward notched type, whereas the male phenotype was scarcely affected. The frequency of notched wings varied with the duration of starvation and the larval age at which starvation began, although no definite effective period could be determined. In experiments in which larvae were kept in a vial containing only filter paper soaked in Ringer's solution (complete starvation), development was delayed considerably, but no marked effect on wing phenotype was seen except that a few flies had strongly notched wings with a V-shaped incision. According to Akita (1955),  $vg^{NP}$  flies have vestigial wings at 30° C. However, the results of a number of experiments in which the effects of starvation and temperature were examined together showed that the starvation effect is covered almost completely by the temperature effect.

Lefevre, G., Jr., and Bartlett, Alan C. Mutant incidence after irradiation of females.

10 successive subcultures, extending over a period of 4 weeks. The incidence of the following visible mutants was determined for each subculture: yellow, white, Notch, and reversions of  $f^{SN}$  to  $f^+$ . Also, sex-linked recessive lethals were studied, testing all daughters of each irradiated female insofar as possible. In this way, clusters of mutations could be detected, and their size and persistence in time could be analyzed. Very large numbers of  $F_1$  offspring have been examined.

Three-day-old  $v f^{SN}$  car (homozygous) *D. melanogaster* females were exposed to 4200-r doses of X-rays and were subsequently mated individually to  $ySi sc^8 f v dl-49 w^a$  males. Each female was transferred through

The trend of mutant incidence with time is best illustrated by the sex-linked-lethal studies. After an initial incidence equivalent to that resulting from irradiation of mature sperm, sex-linked lethals declined in frequency for the first week. The frequency was relatively constant during the second and third weeks, but in the fourth week it increased erratically, in some runs becoming as high as or higher than the initial rate. The visible mutants showed the same general trend, but with considerably less variation in incidence throughout the 4-week period.

The size of mutant clusters tended to be small. Most frequent were

clusters of 5-4 or 7-8 individuals. In only one case, a white mutant, were there more than 8; in that case 9 individuals were found. The method of culturing made it likely that most, if not all, of the mutant eggs were recovered. Only during the first week, before clusters occurred was the fertility of the irradiated females below normal. In the extreme, over 1000 progeny were produced by one female in the 4-week interval.

Further experiments of this sort will be delayed because of a disaster that wiped out the entire fly colony at Salt Lake City just at the end of the current series of tests. New stocks are being obtained as replacements.

Lewis, E. B. Additions and corrections to the cytology of rearrangements.

102-109):

Rearrangement

$C(3)x$  Breakage Points  
Two inversions apparently identical to 3L and 3R Payne.

$T(1;4)B^s$

The break in four is in 102F.

$T(2;3)101$

44B / 83E or F.

$T(2;3)Hn$

54A or B / 76 or 77. In addition there is a deficiency

(associated with the Henna effect) in the region of

66A and B (exact limits not determined).

$T(2;3)p^{Gr}$

57C / 81F.

$T(2;4)d$

55E or F / break in four not determined.

$T(3;4)e$

79E / 102F.

$T(3;4)f$

Insertion of at least seven bands of chromosome four

(bands not identified) into 3L, probably just after

65D1-2.

102-109):

Lewis, E. B. Addition and corrections to the list of mutants in the work of Bridges-Brehme (1944).

Tft: Tufted Since "tufted" (symbol: tuf) is already in use (see DIS-22: 56), it is proposed that the name associated with the symbol Tft be changed from Tufted to Tuft.

Hn<sup>r3</sup>: Henna-recessive-3 New symbol and name proposed for sed, which proves to be allelic to Hn<sup>r1</sup> (and Hn). More extreme, and therefore generally more useful, than Hn<sup>r1</sup>.

sed: sepioaid Name and symbol discarded. Symbol changed to Hn<sup>r3</sup>.

ld: loboid Locus between ca and bv at 102± (instead of 100±).

Lindsley, D. L., and Novitski, E. Influence of the proximal regions of the fourth chromosomes on their meiotic behavior.

Females heterozygous for In(1)sc<sup>8</sup>, f v cv sc<sup>8</sup> or In(1)sc<sup>8</sup>L, EN<sup>R</sup>•Y<sup>L</sup>, y<sup>+</sup> f v cv y and X(Y<sup>L</sup>•)4, y<sup>2</sup> su-w<sup>a</sup> w<sup>a</sup> (the order of the centromere and Y<sup>L</sup> in the last case is unknown) were found to fall into two groups: those that gave haplo-4 progeny

and were presumed to carry one free fourth chromosome (4/0), and those that gave no haplo-4 progeny and were presumed to have two free fourth chromosomes (4/4). Consider what happens when the inverted chromosome separates from the  $XY^{L\cdot 4}$  chromosome in females with one free 4. If the free 4 pairs with and separates from the fourth-chromosome portion of the  $XY^{L\cdot 4}$ , each meiotic product will receive a fourth chromosome. If, on the other hand, the free 4 passes to the same pole as the  $XY^{L\cdot 4}$ , the inverted chromosome must pass to a nullo-4 pole and give rise to a haplo-4 zygote. Perfect disjunction of the free 4 from the  $XY^{L\cdot 4}$  produces no haplo-4 inversion zygotes, whereas random disjunction of the free 4 should render half of the inversion-bearing zygotes haplo-4 and consequently extremely inviable.

Since presumably every zygote produced by the 4/4 females will receive a fourth chromosome from the mother, data from such females provide a control. Furthermore, since  $XY^{L\cdot 4}$  progeny will never be haplo-4, the ratio of inversion progeny to  $XY^{L\cdot 4}$  progeny will give the relative production of inversion zygotes from both 4/4 and 4/0 females. If one assumes complete lethality of haplo-4 zygotes by ignoring all haplo-4 individuals recovered, any decrease in the above ratio in the 4/0 as opposed to 4/4 females indicates something less than perfect separation of a single free 4 from the  $XY^{L\cdot 4}$ . A ratio from 4/0 females that is half the ratio from 4/4 females indicates random assortment of the  $XY^{L\cdot 4}$  and the free 4.

From females of constitution  $y^2$  su-w<sup>a</sup> w<sup>a</sup> bb ( $Y^{L\cdot 4}$ )4 #179-8/In(1)sc<sup>8</sup>, f v cv sc<sup>8</sup> there were 258 sc<sup>8</sup> daughters and 484  $XY^{L\cdot 4}$  daughters for a ratio of 0.553 from 4/0 females, and 638 sc<sup>8</sup> daughters and 690  $XY^{L\cdot 4}$  daughters for a ratio of 0.914 from 4/4 females. The recovery of the inversion from 4/0 mothers was 0.583 that from 4/4 mothers. Similarly,  $y^2$  su-w<sup>a</sup> w<sup>a</sup> bb ( $Y^{L\cdot 4}$ )4 #179-8/In(1)sc<sup>8L</sup>, EN<sup>P</sup>· $Y^L$ ,  $y^+$  f v cv y; 4/0 females yielded 87 sc<sup>8EN</sup> and 157  $XY^{L\cdot 4}$  daughters (= 0.554), whereas  $y^2$  su-w<sup>a</sup> w<sup>a</sup> bb ( $Y^{L\cdot 4}$ )4 #179-8/In(1)sc<sup>8L</sup>, EN<sup>R</sup>· $Y^L$ ,  $y^+$  f v cv y; 4/4 females yielded 968 sc<sup>8EN</sup> and 841  $XY^{L\cdot 4}$  daughters (= 1.153). Here the recovery of the inversion from 4/0 mothers was 0.481 that from the 4/4 mothers.

The fact that a single free 4 fails to pair with and disjoin from the fourth-chromosome portion of an  $XY^{L\cdot 4}$  chromosome suggests that the portion of the fourth chromosome which controls pairing and disjunction is absent from the  $XY^{L\cdot 4}$  chromosome. Since the  $XY^{L\cdot 4}$  chromosome is an induced detachment of an  $XY^{L\cdot 4}$  chromosome the region of the four missing is almost certainly proximal, but its extent is unknown, since sv<sup>+</sup> is the only fourth-chromosome gene identified on the  $XY^{L\cdot 4}$ , and this is the most distal gene on chromosome 4 according to Sturtevant (1951).

Lindsley, D. L., and Sandler, L. The effect of a free heterochromatic X-chromosome duplication on the disjunction of normal fours.

certain of the duplications. It was further noticed that the high incidence of haplo-4 offspring was correlated with high nondisjunction of the attached-X and the duplication, and that the haplo-4 individuals nearly always carried the duplication (39 of 43 cases recorded). One case in which careful counts were made on the Minute progeny was a cross of  $y^2$  w/Dp(1;f)135 females x  $y^S X \cdot Y^L$ , In(1)EN,  $y^2$  B/O males. The progeny included 1684  $y^2$  B males, 416  $y^2$  B

In a series of crosses in which attached-X females carrying different free heterochromatic X-chromosome duplications marked with  $y^+$  or  $y^2$  were crossed with  $y^S X \cdot Y^L$ , In(1)EN,  $y^2$  B/O males, a high incidence of haplo-4 progeny was noticed from females carrying

males, 1978 y w females, 417 y<sup>2</sup> w females, 14 y<sup>2</sup> B M males, 2 y w M females, and 13 y<sup>2</sup> w M females.

The interpretation of these observations is that those duplications that do not have a particularly strong affinity for the attached-X tend, in a proportion of the cases, to pair with and separate from one of the fourth chromosomes. When the remaining fourth chromosome passes to the same pole as the duplication, each of the products receives one four; but when it passes to the opposite pole, that pole gets two fours and the cell that receives the duplication lacks a fourth chromosome. Since a number of different duplications show this effect and since duplications that show the effect definitely carry proximal-X heterochromatin as shown by the presence of  $bb^+$ , it seems likely that these observations provide additional evidence of pairing homology between the X and 4. These observations are similar to those of Gershenson (1940), who found that similar heterochromatic X duplications separate regularly from the third four in triplo-four flies.

Lüers, T. Cyto-architectonic studies in the central nervous system of the adult *Drosophila*.

The structure of the cortex of the central nervous system has been studied in late pupae and young adults of *D. melanogaster* and *D. funebris*. The material, sectioned in series in different directions, has been stained by Nissl's method (Kresylechtviolett). The different cell types, characterized by their size, form, and internal structure, build up typical bilaterally symmetrical patterns. In the cortex of the brain there could be demonstrated eleven main cyto-architectonic regions, some of which are separated from each other by sharp boundaries. An extremely sharp boundary is seen between the protocerebral and the optic lobe. In the cortex of the latter are found the smallest cells of the whole nervous system, forming a homogenous cell area. Nearly all the other areas are characterized by different combinations of the different cell types, especially by scattered clusters of giant cells. In the cortex of the thoracico-abdominal ganglion an adequate arrangement is given. The areas can be classified according to their position in relation to the neuromeres. In this ganglion the biggest cells of the whole system are found between the prothoracic and mesothoracic neuromeres. In respect to the different types of nerve cells the Nissl-method makes possible the best analysis of the internal structure. There are great differences with regard to the nucleus-cytoplasm relation among cells of different size, with a shift from the smallest to the largest in favor of the cytoplasm. This stains dark blue, with a very fine granulation in the giant cells. On the whole, a highly differentiated cellular organization can be established in the cortex of *Drosophila*.

Lüers, H. Examination of the number of active primary germ cells in the late imago of *Drosophila*.

On the basis of results of radiation experiments, H. J. Muller has established that the number of proliferating primary germ cells diminishes in the aging male imago of *Drosophila*, usually to about two in each testis. Mutagenicity experiments were made with 2:5-bis-ethylene-imino-benzochinone-1:4, administered in three days' feeding, to *D. melanogaster* males 1-2 days old. Each  $P_1$  male was tested individually by the Muller-5 technique in ten successive broods, each of three days' duration. It was found that in experiment no. 7 male no. 3 produced a cluster of about 50% lethal mutations (33/80) in broods six to ten, and male no. 30 gave 100% lethal mutations (81/81) in the same broods. The percentages of lethals in these two

males in broods one to five had been 5.6% and 14.6%, respectively. Thus there is the possibility that in both males only one primary cell in each testis had been actively proliferating from brood six on (age of the males at this time, 19-23 days). These cells carried a lethal in the one and no lethal in the other testis in male no. 3, and one lethal in each testis in male no. 30. All lethals of the two clusters will be tested, by locating them through the use of an X-ple stock, in order to check on the expectation of identical lethals in male no. 3 and of two different sorts of lethals in male no. 30.

Lüders, T., and Köpf, H.  
Morphological observations  
on the neurosecretory cells  
of adult D. funebris.

Neurosecretory cells have been demonstrated in many groups of insects. Although in *Drosophila* physiological investigations have been numerous, descriptions of the morphology and distribution of these cells are rare. M. Vogt (1942) observed 4 to 8 fuchsinophilic cells in the pars intercerebralis of the larval brain in *D. melanogaster*. We stained sections of late pupae and adults of *D. funebris* at different ages, by Gomori's method. In the brain it was possible to recognize a main group of at least 20 cells in the pars intercerebralis. Two lateral groups of about three cells each are situated at the transition zone between the protocerebral lobes and the optic lobes. A few isolated large neurosecretory cells are scattered in other parts of the cerebral ganglion, a cluster of two situated in the cortex of the antennal lobes. In the thoracic ganglion several groups of neurosecretory cells can be found, bilaterally placed, adjacent to the neurofiles. All these cells are relatively large. The large and light nuclei are more or less rounded, containing chromonemata and chromocenters and red-colored nucleoli. In respect to the cytoplasm, there can be found two different types of cells in the same clusters, one type dark blue in color and the other dark reddish. In comparison with neurosecretory cells of *Calliphora* and *Musca* stained by the same technique, *Drosophila* showed no distinct granules, but a diffuse coloring resembling colloids. In some cases a large vacuole can be observed in the neurosecretory cells of the thoracic ganglion. Up to now no transport of the secretory products along the axons has been found.

Makino, S., Momma, F., and  
Wakahama, K. Fluctuation of  
predominant species of  
*Drosophila* at Sapporo.

Makino, S., Momma, F., and  
Wakahama, K. Fluctuation of  
predominant species of  
*Drosophila* at Sapporo.

Trappings were made in the University Botanical Garden at Sapporo during every month except the snowfall season (November to April) for three years, 1954-1956. *D. nigromaculata*, *D. auraria*, and *D. transversa* were found to be most common in each year; but their frequencies of occurrence showed variations. *D. nigromaculata* ranked first in 1954 and 1956, but showed a striking decrease in 1955 and 1956. *D. auraria* ranked first in 1955, when it showed the highest frequency of the three years. *D. transversa* ranked third in each year, with slight variations in frequency. The order of frequencies is given below.

(Table on following page.)

Year	Rank			Total flies collected
	I	II	III	
1954	D. nigromaculata (52.68%)	D. auraria (20.65%)	D. transversa (12.94%)	2295
1955	D. auraria (35.50%)	D. nigromaculata (24.25%)	D. transversa (15.00%)	800
1956	D. nigromaculata (23.12%)	D. auraria (21.16%)	D. transversa (14.02%)	3609

Matthews, P. A sex-limited semi-lethal in D. melanogaster

During the course of a series of in-breeding experiments a sex-limited semi-lethal was discovered in a wild-type stock, Hampton Hill. Matings between homozygous males and females gave progenies greatly varying in their sex ratios, generally deviating significantly from an expected 1:1 ratio. Lethality was limited to females, although a few did break through the lethality barrier. Hence the stock could be maintained in a homozygous condition. Progenies of single-pair matings from the lethal stock gave percentages of females varying from 14.8% to 51.4%. Data from a large number of such matings gave an over-all excess of males to females of 2 to 1. Reciprocal matings to normal stocks gave 1:1 sex ratios. Other reported cases of sex-limited lethals in D. melanogaster (Bonnier, 1923; Morgan, 1929; Gowan, 1949; and Bell, 1954) all gave reciprocal differences in sex ratios when the lethal stock was mated to wild-type stocks--excesses of males being observed when the lethal stock was used as female. In three of the above cases the action of the lethal occurred in the egg stage (Morgan, 1929; Gowan, 1949; and Bell, 1954), so that one might assume that the reciprocal difference is in part a reflection of the genetic structure of the female.

The time of action in the present lethal appears to be during the late pupal stages and in the pupal-imago transition period. The exact time of action varies in different cultures. Upwards of 50-to-1 of the dead flies recovered from lethal cultures have proved to be females. These females were characterized by a series of abnormalities affecting the chaetae of the dorsal surfaces of head, thorax, and abdomen; the structure of the dorsal surface of the thorax; and the normal inflation of the wings. The regular pattern of the minor chaetae of the dorsal thorax was disorganized: the posterior scutellars were upright; dorsocentrals either upright or missing; postverticals, verticals, and orbitals missing or arranged in an irregular fashion. The lethal was not without its effect on the males. The wings of the majority of males were slightly upcurved, in extreme cases resembling Curly. Occasionally the same characteristic was noticed in females, the curling being nowhere near as marked as in the males.

An extensive series of salivary-gland studies of the lethal stock has revealed no major inversions or alterations in chromosomes X, 2, or 3. Tests to locate the position of the lethal are at present under way, and a more detailed report will be presented elsewhere.

Meyer, Helen U. Failure of inseminated females to produce fertilized eggs unless additional copulation takes place.

desired composition (autosome to be tested/balancing chromosome). This happens mainly in poorly going, moldy vials, where some of the flies may have died before one attends to selecting the suitable parents for the next generation.

If in such a case only female, but no male, heterozygotes can be obtained --not even at a later date--it is our custom to culture such females for at least one week, assuming that they had been fertilized by brothers which since had died. After this waiting period the vial is checked for collapsed eggs or larvae; only when no sign of fertilization can be found do we remate the female to suitable males of another composition, in order to complete the test for lethals. (We have found that the frequency of lethals in the poorly going cultures is somewhat higher than in the rest of the group which they represent; ignoring such cultures would therefore bias the results.)

Judging from the offspring obtained from females that had laid unfertilized eggs only during the first waiting period, it was found that each must indeed have mated with a brother and contained stored sperm from such a first mating, but could produce fertilized eggs only after being given additional males.

We conclude from this observation that, oftentimes, females do not or cannot utilize stored sperm. The reason for this, we think, may be an insufficient amount of sperm or of glandular secretions--or probably both--delivered at the first copulation. Another possibility may be that the need for polyspermy cannot be adequately met without more sperm.

(This work has been supported by a grant to Dr. H. J. Muller and associates from the American Cancer Society.)

Meyer, Helen U. Frequency of detachments of attached-X chromosomes in the presence of sc.Y<sup>L</sup> or Y<sup>Lc</sup> spontaneously and after irradiation of polar caps.

Since it is known that most spontaneously occurring detachments of attached-X chromosomes are the result of crossing over with the Y chromosome (Kaufmann, 1953; Philip, 1934), it was expected that the type of Y chromosome present would greatly influence the rate of spontaneous detachments. This was confirmed by Parker (1954) for radiation-induced detachments. Comparing several types of females having different Y chromosomes or not having a Y at all, he found that the frequency of exchanges was greater when a two-armed Y chromosome was present in the attached-X females than when the Y was ring shaped or entirely absent.

Even though radiation-induced exchanges most certainly do not take place at the stage of premeiotic crossing over, Parker could confirm that exchanges with the Y chromosome accounted for by far the most numerous cases of detachments if a Y chromosome had been present, or if it was not a ring. In the latter two instances, almost all exchanges involved the fourth chromosome, which always ranks second as a supplier of a new chromosome end for the detached-X chromosome. This was also reported by Abrahamson, Herskowitz, and Muller (1954, 1956) for detachments obtained from irradiated females without a free Y chromosome.

In our experiments we compared the detachment frequency in attached-X females which had either a sc.Y<sup>L</sup> (Crew and Lamy, 1940) or a Y<sup>Lc</sup> (Muller, 1948); both had also been studied by Parker in his comparisons. The sc.Y<sup>L</sup> is V shaped and contains a great amount of heterochromatin, whereas the Y<sup>Lc</sup> is a ring and seems only about half the mitotic length or bulk of the sc.Y<sup>L</sup>. The attached-X chromosomes were of "snoot" type (sc ct<sup>1</sup> oc ptg car. In49sn<sup>x2</sup> ct<sup>1</sup>, In y), and such females with either a sc.Y<sup>L</sup> or a Y<sup>Lc</sup> were mated to males oc ptg.Y<sup>S</sup>/sc.Y<sup>L</sup> or Y<sup>Lc</sup>, respectively.

From such a cross, all daughters should be ct and all sons ct<sup>+</sup>, oc ptg, like the parents. However, if detachment of the attached-X's should have occurred, we would find some non-cut females and ct males, which could then be tested to determine whether they really were the products of detachments.

By far the greatest number of females in our experiment were untreated; a smaller number from both groups had been treated at an early embryonic stage (polar cap stage) with either ultraviolet (300 ergs/mm<sup>2</sup> of mainly 2557 Å), or X-rays (1500 and 2000 r, 200 kvp) applied to the region which then contained the pole cells. Only a few of the X-ray group survived. To check the effectiveness of the treatment, male embryos which had been irradiated with the females were tested for autosomal lethals. We obtained the following results:

Group	Treatment	No. of P females	Confirmed detachments in F <sub>1</sub> females*		Lethals, chrom. 2**	
			No.	%	No. tests	%
sc.Y <sup>L</sup> group	untreated	131	4/8164	.049	1232	.32
	ultraviolet	53	1/3256	.031	1143	5.0
	X-rays	31	-/1512	0.0	754	2.6
Y <sup>Lc</sup> group	untreated	115	-/6307	0.0	1315	.61
	ultraviolet	28	-/1011	0.0	178	8.4
	X-rays	10	-/ 458	0.0	129	0.0

\* No evidence of detachments found in a similar number of F<sub>1</sub> males.

\*\* Found in offspring from brothers of P females, see text.

We see then that our data for the spontaneous rate of detachments agree with the results which Parker obtained in his irradiation experiments; whereas .04% of the F<sub>1</sub> females of the sc.Y<sup>L</sup> group had detached-X chromosomes, none were found in the group having the ring Y. This was no doubt due to the shape of the latter, which allows only double crossovers to survive.

No exceptions at all were found among F<sub>1</sub> males. Parker, who also found many fewer male than female exceptions in irradiated material, attributed it only in part to induced lethals in the detached chromosome portion, but had reason to believe that some of the attached-X's had pre-existing lethals accumulated in regions near the centromere. In our case we expected only half as many male as female exceptions to begin with, since one arm of the "snoot" attached-X chromosome carries a known lethal (ct<sup>1</sup>), and with the low number of female exceptions in our untreated material an explanation on statistical grounds might be sufficient. Since the frequency found from F<sub>1</sub>

females alone is no doubt closer to the true detachment frequency than if based on the sum of the males and females; only the figures for females are listed in the table.

We further see from the data in the table above that, apparently, the frequency of detachments is not increased by irradiating the future germ cells at such an early stage. We do not believe that this is a true result, for the following reasons: (1) both X-rays and ultraviolet are known to increase non-meiotic crossing over, especially in heterochromatic regions; (2) we had evidence that the treatment must have affected the germ cells from the increase in autosomal lethals obtained from corresponding males, except for the X-ray-treated  $\gamma$ LC group; (3) we found in a more recent experiment, also using females, but designed to discover a different type of detachment (of translocated parts attached to free X chromosomes), that detachments can be obtained by either ultraviolet or X-ray treatment of early germ cells. Therefore we are inclined to think that there probably were cases of detachment caused by the treatments, which we were unable to detect. The reason might well be, as suggested by Muller, that in those pole cells in which detachment of one chromosome arm had occurred at this early stage of germ-cell development, a segregation of the arms followed, so that the cells became genetically male and failed to furnish properly functioning germ cells and nurse cells within a female gonad.

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Milani, R. Errandation.

The last issue of DIS (DIS-29: 139)

included a contribution by the present author with the title, "Effect of natural selection on the full expression of the gene countercoiled (cc)." This note did not mention the organism involved, which was Musca domestica. It seems desirable to clarify this point, because a similar mutant type has been detected in D. melanogaster.

Milani, R. Countercoiled genitalia in D. melanogaster.

Toward the end of 1951, in scoring the  $F_2$  of a wild D. melanogaster female it was found that some males had the hypopigium rotated out of its normal position. These

males, sexually active and otherwise perfectly normal, were sterile through a mechanical impediment to copulation. A line was started from single-pair matings of sibs of the abnormal males. Observations carried through a few generations provided evidence of monofactoriality for the abnormal rotation, which behaves as a recessive. Dissection of preserved specimens has recently shown that this abnormality involves a counterclockwise looping of the sperm duct around the rectum. A search among normal strains having a common origin with the one in which this abnormality was first observed revealed some counterclockwise males (homozygous?; about 2%). The morphological effect of this mutant type is closely similar to that of the mutant of Musca domestica which I called "countercoiled genitalia. However, it appears that in D. melanogaster the countercoiling of the hypopigium can very rarely be fully accomplished through  $360^\circ$ , whereas in some strains of the housefly the countercoiling is usually fully accomplished in nearly all males.

Milani, R. Housefly genetics. Evidence of linkage between two markers and of male crossing over in the housefly.

However, the segregation ratios do not fit the expectation, owing to a great shortage in the double-recessive class; there are gross differences between lines. Backcross tests have shown a rather regular 40% crossover rate when the tested heterozygous flies were females; when the tested flies were males, close linkage has been found in most families, but in a few families crossover classes have been found. Comparison of the female crossover rate (about 40%) with the recombination values observed among intercrossed  $F_2$  flies suggests that crossing over between bwb and div can reach 10% in the males. All the gynandromorphs found so far (7) showing mosaicism for these two markers had a topographic distribution of marked tissues in keeping with the nature of the original cross.

Miller, D. D. "Choice matings" involving D. athabasca from Wyoming, Michigan, and New York.

in all the possible combinations of Wyoming (Jackson Hole), Michigan (University of Michigan Biological Station), and New York (Cold Spring Harbor). In all cases the frequencies of insemination of the two kinds of females were significantly different. Wyoming males inseminated 5/90 (6%) Michigan and 39/100 (39%) New York females; 5/37 (3%) Michigan and 67/90 (74%) Wyoming females; and 35/100 (35%) New York and 85/100 (85%) Wyoming females. Michigan males inseminated 79/102 (79%) Michigan and 1/101 (1%) New York females; 68/97 (70%) Michigan and 7/98 (7%) Wyoming females; and 0/106 (0%) New York and 5/101 (5%) Wyoming females. New York males inseminated 0/101 (0%) Michigan and 62/102 (61%) New York females; 0/37 (0%) Michigan and 15/87 (17%) Wyoming females; and 72/100 (72%) and 24/99 (24%) Wyoming females. It may be seen that interlocality insemination frequencies involving Michigan D. athabasca were consistently lower than those involving Wyoming and New York strains, with almost complete sexual isolation between Michigan and New York. However, frequencies of insemination between Michigan and Wyoming were appreciably higher than those observed the year before in "no-choice" combinations of D. athabasca from these localities (Miller, DIS-29). Additional "no-choice" matings of Wyoming and New York D. athabasca yielded abundant fertile offspring. These results help reconcile the findings of Novitski (1946), who did not report sexual isolation between western and eastern D. athabasca, with those of Miller (DIS-29), who reported a high degree of sexual isolation between western (Wyoming, North Dakota, and western Ontario) and eastern (Michigan) D. athabasca.

Miller, D. D. Geographical variation in copulation time in D. athabasca.

Lake) (3'57"-15'29", Miller, DIS-29); short in strains from Michigan (Cheboygan) (1'6"-2'0", Miller, DIS-29), New York (Cold Spring Harbor), and New Jersey (Princeton) (1'12"-1'48", Miller, DIS-25). A few copulations have since been observed in recently derived (summer of 1956) D. athabasca strains, and the following durations have been determined: Iron River, Wisconsin,

The markers divergent (div) and brown body (bwb) of the housefly can be found combined in a double-recessive class in the  $F_2$  of crosses in which they have been introduced one from each parent.

These "choice matings" were made with 7-8-day adult flies, and lasted 3 to 4 days. Males were combined with a mixture of females from two different localities,

in all the possible combinations of Wyoming (Jackson Hole), Michigan (University of Michigan Biological Station), and New York (Cold Spring Harbor). In all cases the frequencies of insemination of the two kinds of females were significantly different. Wyoming males inseminated 5/90 (6%) Michigan and 39/100 (39%) New York females; 5/37 (3%) Michigan and 67/90 (74%) Wyoming females; and 35/100 (35%) New York and 85/100 (85%) Wyoming females.

Michigan males inseminated 79/102 (79%) Michigan and 1/101 (1%) New York females; 68/97 (70%) Michigan and 7/98 (7%) Wyoming females; and 0/106 (0%) New York and 5/101 (5%) Wyoming females. New York males inseminated 0/101 (0%) Michigan and 62/102 (61%) New York females; 0/37 (0%) Michigan and 15/87 (17%) Wyoming females; and 72/100 (72%) and 24/99 (24%) Wyoming females. It may be seen that interlocality insemination frequencies involving Michigan D. athabasca were consistently lower than those involving Wyoming and New York strains, with almost complete sexual isolation between Michigan and New York. However, frequencies of insemination between Michigan and Wyoming were appreciably higher than those observed the year before in "no-choice" combinations of D. athabasca from these localities (Miller, DIS-29).

Additional "no-choice" matings of Wyoming and New York D. athabasca yielded abundant fertile offspring. These results help reconcile the findings of Novitski (1946), who did not report sexual isolation between western and eastern D. athabasca, with those of Miller (DIS-29), who reported a high degree of sexual isolation between western (Wyoming, North Dakota, and western Ontario) and eastern (Michigan) D. athabasca.

As has already been reported, copulation in D. athabasca is long in strains from Wyoming (Jackson Hole), North Dakota (Minnewaukan), and western Ontario (Cedar

Lake) (3'57"-15'29", Miller, DIS-29); short in strains from Michigan (Cheboygan) (1'6"-2'0", Miller, DIS-29), New York (Cold Spring Harbor), and New Jersey (Princeton) (1'12"-1'48", Miller, DIS-25). A few copulations have since been observed in recently derived (summer of 1956) D. athabasca strains, and the following durations have been determined: Iron River, Wisconsin,

4'56"; Iron Mountain, Michigan, 1'25" and 1'26" (these strains kindly provided by Dr. H. D. Stalker of Washington University); Algonquin Park, Ontario, 1'17", 1'30", 1'39", 2'16", and 2'42"; Gatineau Park, Quebec, 1'11", 1'24", 1'26", 1'56", 1'59", and 4'11"; Ste. Anne de Bellevue, Quebec, 1'35" and 1'59"; and Laurentides Park, Quebec, 5'56", 7'18", 7'20", 7'59", 10'7", and 20'56". (As before, all those have been first copulations of week-old flies.) The results show that the long-copulation-time characteristic extends into the east in the northern part of the known range of this species, with some localities (Algonquin and Gatineau Parks) having both short copulation times and copulation times longer than previously reported for eastern strains.

Three copulations have been observed between "short"- and "long"-copulation strains: New York female by Wyoming male, 4'18"; Wyoming female by New York male, 1'3" and 1'11". These results are at least consistent with male determination of copulation time, such as was reported by Merrell (1949) for inbred *D. melanogaster* strains. Also observed have been a few copulations of hybrids between "short" and "long" strains:  $F_1$  (N.Y. female by Wyo. male), 1'13", 2'2", 2'16", 2'25", 2'26", 2'50", and 3'9";  $F_1$  (Wyo. female by N.Y. male), 5'19". These results show that hybrids may have copulation times intermediate between those characteristic of the parent strains. Work is in progress to augment the observations of copulation time in the new strains and between "long" and "short" strains and their hybrids.

Mather, W. B. Genetic relationships of four *Drosophila* species from Australia.

(The following is the summary of a paper to be published in a forthcoming University of Texas Publication.) By breeding tests it has been established that: (1)

*D. serrata* Malloch is a biological species distinct from *D. kikkawai* Burla. (2) The fly previously recorded as *D. takahashii* Sturtevant from Australia is in fact a new species, *D. pseudotakahashii*, morphologically distinguishable from *D. takahashii* by two features of the internal male genitalia, which are controlled multifactorially. (3) The synonymy of *D. levis* Mather with *D. bryani* Malloch, previously established on morphological grounds, is confirmed biologically. (4) *D. versicolor* Mather from Australia is synonymous with *D. buzzatii* Patterson and Wheeler, and contains an inversion previously recorded only from Lebanon.

Mather, W. B. Relationships between species groups of the *Pholadoris* subgenus.

(The following is the summary of a paper to be published in a forthcoming University of Texas Publication.) The failure of hybridization between species groups

of the *Pholadoris* subgenus, established on morphological grounds, supports the biological reality of these groups. However, sexual isolation is incomplete between the *levis* and *mirim* groups, between the *maculosa* and *victoria* groups, and between the *maculosa* and *coracina* groups, indicating the close biological relationships of the crossmating groups.

Mitchell, D. F. Persistence of a *sepia* allele in an inbred line.

In a strain derived by sib mating from the offspring of a single female from a wild collection, an allele of *se* has persisted for 20 generations of brother-sister

matings. The allele was presumably present in heterozygous condition in the wild female. The strain has been derived by making four single-pair sib

matings each generation, and selecting wild-type individuals for the next generation from the most productive, or one of the most productive, cultures. Thus, inbreeding and selection for viability under the culture conditions has been involved. Approximately one-half of the cultures contain surviving flies which are heterozygous for the *se* alleles. Homozygotes for *sepia* are not necessarily produced, or do not survive in every generation. It appears, therefore, that the allele has been maintained in the line through the superior fitness of the heterozygotes, and that, under the culture conditions, the homozygous wild type is significantly inferior.

Momma, E. Spermatogenesis in  
*D. lacertosa*.

This is a new species described by Okada (1956), and is rather common in the forests of Hokkaido. Spermatogenesis in

the newly emerged larva was investigated with both fixed and living materials. In material fixed with modified PFA 3 or Champy, the chromosomes make their clear appearance after Heidenhain's iron-hematoxylin staining. Material stained with Regaud's iron-hematoxylin shows the mitochondria with their characteristic features. The mitochondria differ slightly in shape from those observed in the living material, but Golgi bodies or dictyosomes are nearly identical with those studied in Champy material. Successive stages ranging from the maturation of germ cells to spermiogenesis were observed in living cells by phase-contrast microscopy. The cell body at metaphase I is very large in size (40-50 micra in diameter), showing the smaller spindle body (10-15 micra in diameter). Through the course of spermatogenesis the behavior of the mitochondria could be traced in the living state.

Morita, T. Tyrosinase activity and free tyrosine content in some strains of *D. virilis*.

Tyrosinase activities and free-tyrosine contents of larvae and pupae of a wild and two mutant strains were compared with one another. The ebony mutant has a

darker color both in the puparium and in the imaginal integument than the wild type, whereas the yellow mutant has a lighter color in both respects. Tyrosinase activity was measured by the manometric technique. In mature third-instar larvae, tyrosinase activity of ebony was higher and that of yellow lower than that of the wild type. Tyrosinase activity of pupae after 24 hours of pupation was remarkably lower than that of larvae of any kind. The estimation of free-tyrosine content was carried out by the method reported in DIS-29. In mature larvae, free-tyrosine content of ebony was clearly higher than that of wild type or of yellow, but no difference could be detected between the latter two. Free-tyrosine content was reduced remarkably in pupae, and this reduction proceeded gradually during the pupal stage. However, the free-tyrosine content of yellow was maintained at a higher level than that of the others. Thus, the differences in degree of puparium pigmentation between wild, ebony, and yellow strains seem to be due to both tyrosinase activity and tyrosine content.

Muller, H. J. Another entire inversion formed by opening of a ring X.

The X chromosome in our stock b85 (b87 in DIS-29), which had originally been of the ring structure designated as  $X^{c2}$  (closed X of Beadle, 1934), has proved now to be

an open X containing an inversion (InEN2) of the entire euchromatic region. Its crossover properties are like those described by Novitski (DIS-23: 94) for the entire inversion (InEN) obtained by him as a result of the opening of

$X^c$  (closed X of L. V. Morgan). This information was not obtained until our present stock list had been submitted to DIS-30. Users of stocks designated as  $X^{c2}$  in this list are warned that any such stock may contain an open X instead of a closed one. Whether the new open X carries pieces of Y or of IV has not yet been determined. Like  $X^{c2}$ , but unlike Novitski's opened X, it contains the normal allele of yellow in its main euchromatic region.

Muller, H. J., and Herskowitz, I. H. Reciprocal and half-translocations with a rod-X chromosome produced by X-raying sperm and oocytes.

the retention of a viable eucentric half-translocation. Unexceptional virgin  $F_1$  females (whose maternal chromosome carried inversions in addition to y) were mated individually to y males, and the  $F_2$  examined. Of 1949  $F_1$  females tested, 22, or 1.1%, carried a paternal X which had undergone a reciprocal translocation of such a kind that one or both half-translocations derived from it were separately viable. Eleven of the 22 were analyzed; of these, 7 were shown to be X-IV's, 1 was X-III, 1 was X-II, and 2 were X-II-III's.

Thus there were among the  $F_1$  about 4 times as many reciprocal translocations as there were all types of gross rearrangement (and point mutation) which became viable aneuploids before advanced cleavage stages. This must mean that if centric unjoined fragments are frequent these usually cause death, and that such fragments once joined are relatively infrequently lost in early development, at least when the breaks are produced in sperm. Of the reciprocal translocations identified, 64% (7/11) were X-IV, a value not significantly lower than the 84% of the half-translocations identified which were shown to be X-IV by similar tests, after irradiation of attached-X's in the absence of a Y (Arehanson, Herskowitz, and Muller, 1956). The preponderance of X-IV's in both studies is attributed largely to the relatively high viability of the aneuploid of IV, but proximity of the parts undergoing exchange may also play a role here.

Either 3600 r or 4250 r were delivered in a concentrated treatment to females homozygous for y  $sc^5.Dp$   $sc^{VI}$   $y^+$ , which were then crossed to y  $sc^{SI}$  B In49 v/Y $^+$  males. From eggs oviposited within 4 days after irradiation, there were in  $F_1$ , for the respective treatments,  $0.58 \pm 0.13\%$  (21/3597) and  $0.67 \pm 0.072\%$  (36/12,931) exceptional individuals, representing the frequency of  $y^+$ -deficient half-translocations and/or deficiencies of  $sc^{VI}$   $y^+$  of the maternal X present in mature eggs. Of 82 exceptions tested, 59, or 46%, were proved X-IV half-translocations. This is in agreement with the 33% and 46% of all half-translocations tested which were proved to be X-IV after irradiation of sperm containing the same X chromosome and irradiation of attached-X's in the absence of a Y, respectively. Phenotypically unexceptional  $F_1$  virgin females were mated individually to males like the  $F_1$  fathers, and each  $F_2$  culture examined for  $y^+$ -deficient half-translocations. For the respective doses, the frequencies obtained were  $< 0.079 \pm 0.08\%$  ( $< 1/1263$ ) and  $0.031 \pm 0.03\%$  ( $1/3255$ ) for reciprocal translocations among  $F_1$  females whose  $y^+$ -deficient half-translocation (obtained in  $F_2$ ) proved viable.

If all half-translocations in mature eggs were produced by the sorting into the polar bodies of the other part of a reciprocal translocation produced in the oocyte, then there could be, among the eggs producing the  $F_1$ , no more

Irradiated (4000 r) y  $sc^5.Dp$   $sc^{VI}$   $y^+$ /Y $^+$  males crossed to females homozygous for y in separate X's gave in  $F_1$  13  $y^+$  males and 15 y females among 10,956 offspring, a total of 0.26%, which were produced by intra-X rearrangements, point mutation, or

than three times as many half-translocations of a given type as reciprocal translocations producing the same type of half-translocation. However, the frequency of these reciprocal translocations is significantly less than one-third the frequency of half-translocations detected as such in  $F_1$ , even without correcting for the fact that the experimental procedure favored the detection of a reciprocal translocation giving a  $y^+$ -deficient half-translocation. This result must mean that half-translocations derived from irradiated oocytes do not always (or usually) arise from reciprocal translocations, but frequently result from two-break events in which only one eucentric exchange union occurred, the other centric piece having been cast off into a polar body unjoined. Accordingly, the reverse situation probably happens equally frequently, in which the centric piece that has joined in a eucentric half-translocation is discarded in a polar body while the unjoined centric piece is retained in the mature egg. This would make a significant contribution to  $\gamma$ -ray-induced egg mortality.

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Nicoletti, B., and Solima, A.  
Differential fitness in D. melanogaster populations.

Fecundity, hatchability, fertility, and rate of development have been studied in Oregon-R, in Oregon inbred (370th brother-sister matings), in a population from the wild (Perugia), and in reciprocal crosses between Perugia and Oregon-R strains, as measurements of Darwinian fitness. It has been found that fitness is inversely related to the degree of inbreeding. The population from the wild shows the highest values of fitness, the Oregon inbred the lowest, and the Oregon-R strain intermediate values. The flies obtained from reciprocal crosses exhibit a high degree of heterosis, whose expression is related, however, with the strain used as female parent.

Nicoletti, B., and Solima, A.  
Sexual selection on D. melanogaster.

Experiments on sexual selection in D. melanogaster have been continued, using Oregon-R and  $ss^a$  strains. When placed in competition with the wild type, the mutant males show lower sexual activity and the mutant females seem to be less sensitive to the male courtship. The experiments made suggest that in intraspecific crosses, when it is possible to obtain evidence of sexual selection, such selection depends not only on the choice made by females, but also on differences in sexual activity of males. Thus, in a given population, sexual selection depends in part upon which males are able to reach most promptly the necessary stimulus for mating, and thereby realize the copulation.

Holte, D. J. Segregation of modifiers.

The quantitative differences, reported previously, in the red and brown eye pigments of different South African strains of D. melanogaster, were studied further by outcrossing three strains, two from the same locality but collected during different years and the third from a widely separated area. The crosses were Inhaca-3 x Inhaca-1, Inhaca-3 x Inhaca-2, Inhaca-3 x Graaff-Reinet-2. From the  $F_2$  of each cross, 50 fertilized females were taken at random to establish inbred lines over a period of ten generations. From these lines 10 were retained of each group

of 15, and these were tested quantitatively for eye pigments. The amount of red pigment differed by about 97% between the highest and lowest lines, and the amount of brown pigment by 58% between the highest and lowest lines for this pigment, the variation of pigment amount in the various lines being differential for the two pigments.

The two ranges of variation for the 50 inbred lines, due to segregation, are fairly continuous but show certain groupings. For the red pigment the number of groupings could be accounted for by the segregation of 10 pairs of modifying genes, and for the brown pigment the number of groupings could be accounted for by the segregation of 7 pairs of genes. There is no correlation between the amounts of red and brown pigment in this series of inbred lines, and it thus appears that the two pigments are influenced independently by two series of polygenes.

The conclusion is that South African wild-type strains are heterogeneous for various combinations of two series of modifiers which are segregating in various populations.

Novitski, E. The possibility of another cytoplasmically inherited factor in D. melanogaster.

847 females and 1 male. Subsequent tests established the following points. The aberrant sex ratio is obtained only when males from certain stocks are mated to these females, the off ratio being obtained also when  $X^{c2}$  males from the same stock from which the female was derived are used, and not when males from Canton-S;  $y\ v/w^a\ v$ ,  $tra/Cx$  and  $y\ w^a\ cv\ v\ f$  stocks are used. Daughters of a female giving an aberrant ratio mated to a "sensitive" male may behave like their mother, may give a normal ratio, or an intermediate ratio, but in general they all behave similarly, suggesting some kind of maternal effect. Because of the presence in these females of an attached-X, the distinction between a cytoplasmic factor and the presence of a factor on that attached-X cannot be made with certainty and awaits the analysis of detachments occurring in lines giving the aberrant ratio. Ordinarily the possibility of cytoplasmic inheritance would be considered quite unlikely, but it is considered more so here because this kind of disturbance in sex ratio has been shown by Magni to be cytoplasmic in D. bifasciata.

Okada, T. The relation between wing indices and wing length.

of the other three indices is inversely proportional to, the wing length. In some species a female usually shows a costal index larger than that of a male of the same species, even when the male and female have the same wing length.

Oshima, C. Studies of DDT resistance in D. melanogaster from the viewpoint of population genetics.

In a set of 25 pair matings of males carrying a specially derived XY chromosome provided by D. L. Lindsley, to females carrying an attached-X, homozygous for  $y$  and  $w$ , and  $sc^8.Y$ , the progeny consisted of

847 females and 1 male. Subsequent tests established the following points. The aberrant sex ratio is obtained only when males from certain stocks are mated to these females, the off ratio being obtained also when  $X^{c2}$  males from the same stock from which the female was derived are used, and not when males from Canton-S;  $y\ v/w^a\ v$ ,  $tra/Cx$  and  $y\ w^a\ cv\ v\ f$  stocks are used. Daughters of a female giving an aberrant ratio mated to a "sensitive" male may behave like their mother, may give a normal ratio, or an intermediate ratio, but in general they all behave similarly, suggesting some kind of maternal effect. Because of the presence in these females of an attached-X, the distinction between a cytoplasmic factor and the presence of a factor on that attached-X cannot be made with certainty and awaits the analysis of detachments occurring in lines giving the aberrant ratio. Ordinarily the possibility of cytoplasmic inheritance would be considered quite unlikely, but it is considered more so here because this kind of disturbance in sex ratio has been shown by Magni to be cytoplasmic in D. bifasciata.

It was found that among the individuals of a drosophilid species or among the various species of the genus *Drosophila* the costal index is roughly proportional to, and each

of the other three indices is inversely proportional to, the wing length. In some species a female usually shows a costal index larger than that of a male of the same species, even when the male and female have the same wing length.

The object of this study was to investigate changes in DDT resistance in populations of D. melanogaster by natural selection. Three wild strains and a mutant strain were used in the experiments: Hikone, which was

highly resistant to DDT; Kanmurijima, slightly resistant; and Canton-S, highly susceptible. A mutant strain which had two recessive marker genes, *sca* and *ssa<sup>a</sup>*, on the second and third chromosomes, respectively, was used as a tester.

Two kinds of analytic populations were prepared and cultured in population cages. One had heterozygous second chromosomes of one of the wild strains and the tester strain, homozygous third chromosomes having the *ssa* gene, and the X chromosome of the tester. The other had homozygous second chromosomes carrying the *sca* gene, heterozygous third chromosomes of one of the wild strains and the tester strain, and the X chromosome of the tester.

After 150 days of culture, these analytic populations were mixed and synthetic populations were made. These populations were cultured in population bottles. Mortality of offspring of several pairs, removed from a population, was measured by exposing for 24 hours to a filter paper impregnated with a DDT concentration of 25  $\mu\text{g}/\text{cm}^2$ . The change in mortality (300 flies tested) of each analytic and synthetic population was investigated during certain generations.

From a statistical analysis of the results it was concluded that the effect of the dominant factor on the second chromosome of the Hikone strain was highest and that of the Canton-S strain was lowest, and that there was a positive interaction between dominant factors on the two major autosomes of each wild strain.

A marked variance in mortality was recognized in the analytic population derived from the Kanmurijima strain. The level of DDT resistance of each synthetic population approached that of the original resistant strain. The effects of resistant factors on each autosome were detected, but the interaction between them was extremely limited. From these results, it is suggested that the dominant factors would gradually be eliminated and, on the other hand, the recessive factors would accumulate in a population by natural selection.

Oshima, C., and Hiroyoshi, T.  
Genetical analysis of DDT and nicotine sulfate resistance in *D. virilis*.

Genetical analyses were performed. The dominant genes responsible for the DDT resistance were found to be located on the second and fifth chromosomes, respectively. The statistical analysis showed that the main effects of the two chromosomal factors were almost equivalent, and that their interaction was positive.

The degree of DDT and nicotine sulfate resistance in various wild and mutant strains of *D. virilis* was determined. Using the Hikone strain, the most highly resistant to both insecticides, genetical

The dominant genes relating to nicotine sulfate resistance were found to be located on the second and fifth chromosomes, respectively; but the main effect exerted by the former was greater than that of the latter and their interaction was not significant. From these experiments it could not be discovered whether or not the genes for resistance to the two insecticides are the same, but it may be assumed that these genes (if there are more than one) might have some common physiological reactions to both insecticides.

It is of significance to an understanding of the processes of evolution

that DDT and nicotine sulfate dominant resistance genes have been located on the homologous chromosome elements of D. virilis and D. melanogaster.

Oster, I. I. A new crossing-over suppressor in chromosome 2 effective in the presence of heterologous inversions.

With a view toward finding a more effective crossing-over suppressor in the second chromosome than the combination of Curly (Cy) and its two large paracentric inversions, one in each arm of the second chromosome, which occasionally undergoes single or double crossing over in the centromeric region and double crossing over in the right arm, we irradiated males containing  $dptxI$  Cy, InL pr  $cn^2$  InCyR with 4000 r. Following a genetic scheme suggested by Muller, the treated males were mated to virgin non-Curly females containing InCyR. The  $F_1$  females were bred individually, and Curly flies from crosses which gave a reduction of recombination in the right arm of the second chromosome were saved for further examination. Such cases were subsequently tested for their effect on crossing over in combination with a normal second chromosome but in the presence of the inversions in the third associated with Moiré and/or  $sc^{S1}$  In49  $sc^8$ . These tests indicated that one of our newly induced inversions, which probably contains a pericentric inversion in addition to InCyL and InCyR, effectively reduces crossing over throughout the length of chromosome 2 even when heterologous inversions are present. This chromosome, containing the complex of inversions which we are designating Cy, InS05, is viable when heterozygous, contains two dominants with lethal recessive effects,  $dptxI$  and Cy, thereby enabling one to use it in methods utilizing Muller's "criss-cross lethal" technique, and should prove useful in breeding schemes involving inversions in the other chromosomes.

(This work has been supported by a grant to Dr. H. J. Muller and associates from the Atomic Energy Commission, Contract AT (11-1)-195, and by a postdoctoral fellowship from the National Science Foundation.)

Oster, I. I. A stock ("Taxy") for detecting translocations of the autosomes and the X and Y chromosomes.

In order to be able to detect not only whole or partial losses of the X or Y, sex-linked lethal mutations, but also translocations involving the autosomes and the X or Y in the offspring of the same treated individuals, a new breeding scheme was worked out. In practice, untreated or treated males of the composition  $Y:bw^+$  /  $y sc^4 B InS w^r sc^8$ ;  $cn bw$  are crossed to females of Bloomington stock j100, composed of  $sc^8.Y / y In49 sn^{X2} B^M l / y oc lz.Y^S$ ;  $twl bw$ ;  $st^{54i}$  females and  $sc^8.Y / y sn oc$ ;  $twl bw$ ;  $st^{54i}$  males, designated "Taxy" (to denote a stock for detecting translocations involving the autosomes and the X or Y). Males containing either  $sc^{S1}$  or  $sc^4$  can be mated, depending upon the main purpose for which one is using the scheme. That is, if one is chiefly interested in detecting lethals and translocations it is better to use males from the stock containing a  $y sc^4 B InS w^r sc^8$  chromosome, since this is more viable than  $y sc^{S1} B InS$ ; whereas if one is mainly interested in detecting losses and partial losses of the X or Y and translocations, it is better to use males containing a  $sc^{S1}$  chromosome since this is better than the  $sc^4 sc^8$  chromosome, which being deficient for the proximal heterochromatic region, undergoes a high rate of spontaneous nondisjunction. The "Taxy" stock is only partially balanced and should be maintained by selection of  $y^+ sn^+ oc^+$  females and  $y^+ sn oc$  males. Regardless of which type of male is crossed to the "Taxy" females, the  $F_1$

offspring can be scored for whole or partial losses of the X or Y, and Bar-eyed females which contain an X derived from their fathers can be tested for sex-linked lethal mutations by being crossed to non-Bar males and looking for the absence of Bar (B) males in the next generation. By virtue of its having been supplied with inversions in one X chromosome and a  $sc^8.Y$ , crosses of the "Taxy" females give rise to two classes of males in the  $F_1$ , those receiving a treated Y from their fathers ( $y$  non-Bar) and those receiving a treated X from their fathers ( $y^+ Bar$ ). Thus, individual matings of the former males to  $y$ ;  $twl\ bw$ ;  $st^{54}i$  virgins, which will detect translocations involving the Y, and of the latter males to  $sc^8.Y / y\ f:=$ ;  $twl\ bw$ ;  $st^{54}i$  virgins, which will detect translocations involving the X, and looking for the absence of one or both classes of recombinants involving any of the three pairs of markers ( $y$  vs.  $y^+$ ,  $twl$  vs.  $twl^+$ , and  $st$  vs.  $st^+$ ), considered two pairs at a time, will indicate the presence of a translocation between the chromosomes with those markers or their alleles. The use of two stocks for testing for either X or Y translocations will enable one to carry out retests by repeating with the  $F_2$  crosses like those of the  $F_1$  of cases which yield insufficient flies to determine whether or not a translocation is present. In addition, this scheme should allow one to detect many more translocations of the Y, which are sometimes sterile if they include position effects on the fertility genes of the Y<sup>8</sup>, because one-half of the males bearing such altered Y's are here rendered fertile since they are supplied with a Y<sup>8</sup> from their mothers.

(This work was supported by a grant for work of Dr. H. J. Muller and associates from the Atomic Energy Commission, Contract AT (11-1)-195, and by a postdoctoral fellowship from the National Science Foundation.)

Pipkin, Sarah B. Balanced polymorphism in D. lebanonensis.

Populations of D. lebanonensis in four different areas in the Lebanon Mountains were found to be polymorphic for an autosomal dominant mutant determining a light scutellum and light area extending anteriorly and laterally as far as the level of the anterior dorsocentral bristles. About half of each of the natural populations showed the mutant "Spot," and about half was homozygous for its recessive allele, "non-Spot" or full color. The dominant mutant "Spot" maintains itself in about half of the populations of crowded culture bottles, according to counts made of the Beirut strain. This is then a new case of balanced polymorphism in Drosophila. In pair matings, D. lebanonensis individuals heterozygous for "Spot" derived from the Beirut strain, when crossed with the closely related D. victoria (Utah strain), which is homozygous for "non-Spot," gave 135 "Spot" and 144 "non-Spot" individuals. Preliminary tests with population cages indicate that a model similar to the one used by Da Cunha (1949) must be used in further studies of this species, since the larvae are extremely active and migratory.

Poulson, D. F. Differences with regard to copper toxicity in ebony strains.

An investigation of a series of ebony alleles with regard to their response to copper concentrations in the medium clarifies an apparent contradiction between my findings and those of Kikkawa, Ogita, and Fujito (DIS-28). The standard ebony strain (e), while showing lower viability than wild strains, will survive at all concentrations at which Ore-R can survive. On the other hand, the ebony-11 strain (e<sup>11</sup>) is extremely sensitive to added copper and does not survive at the higher copper concentrations used. Sooty (e<sup>S</sup>)

appears intermediate in this respect. Whether these are strain or allelic differences is being thoroughly investigated.

Poulson, D. F. Spontaneous reverions of ebony, vermillion, and apricot.

These a non-ebonv v, bw, ey<sup>2</sup> strain was derived, which when crossed to the original v, bw, e, ey<sup>2</sup>, gives typical wild-ebonv segregation. The reversion is being subjected to intensive study with regard to its copper-accumulating properties.

In the same v, bw, e, ey<sup>2</sup> stock a single female was found in which a symmetrical brown-pigmented area, including about one-quarter of the ommatidia, was present in one eye. This is the first time such a patch has even been observed in this stock, which has been rather closely examined over a considerable period of time. This clearly is a case of somatic reversion of vermillion. Another case interpretable only as a somatic back-mutation appeared in a single male of our M-5 stock. In this individual a symmetrical red-pigmented patch was present in one eye and presumably represents a change at the apricot locus. No such patches have been previously observed in any of our apricot-carrying stocks.

Raimondi, G. Lethality in a tumorous stock of D. melanogaster (tu-So<sup>C</sup>).

an incidence of 53%. Counts of surviving larvae hatched from a fixed number of eggs (300 for each experiment) have shown without exception a death rate of 23% at a stage corresponding to a length of 2.5-3.5 mm. A certain amount of death has been detected also in the egg. Corresponding lethality fails to occur in the So<sup>C</sup> stock without tumors. The relation between production of the melanotic mass and cause of death remains obscure.

Rasmussen, E. Resistance to organo-phosphorous insecticides.

been reported previously. It was possible to increase the resistance by means of selection, which was performed during development by adding increasing amounts of para-oxon to the substrate. By now the resistant strains tolerate a concentration of 20-30 ppm, whereas two sensitive strains used as controls tolerate less than 1 ppm and have resisted all attempts to increase their tolerance. Insecticides of this type are known as cholinesterase inhibitors, and the cholinesterase activity of the flies has been determined to find out if the differences in resistance are due directly to differences in that enzyme system. This is not the case, as all the strains have identical enzyme activity. Radioactive parathion marked with S<sup>35</sup>, has been used to study the uptake of the insecticide. This has been accomplished by means of the interrelations between radioactivity, enzyme activity, and LD-50. It was found that the LD-50 of the sensitive strains corresponds to a radioactivity of 30-35 c/min, whereas the resistant strains have a radioactivity of 125-300 c/min, when LD-50 is reached. All strains coincide in their enzyme activity,

In a stock marked by the character Sine oculis (So<sup>C</sup>), melanotic masses have been found with an incidence of 5% in the adult stage, whereas larvae 2-2.5 mm long show

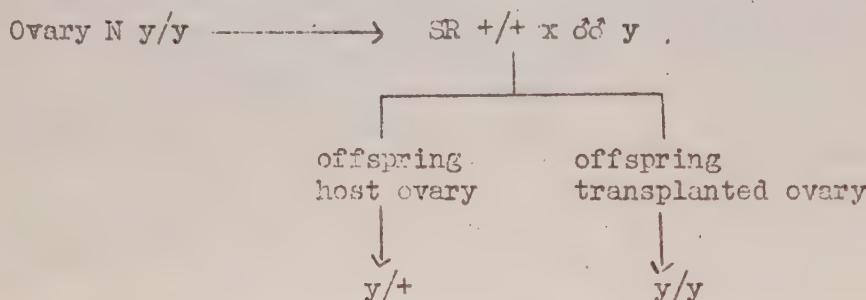
an incidence of 53%. Counts of surviving larvae hatched from a fixed number of eggs (300 for each experiment) have shown without exception a death rate of 23% at a stage corresponding to a length of 2.5-3.5 mm. A certain amount of death has been detected also in the egg. Corresponding lethality fails to occur in the So<sup>C</sup> stock without tumors. The relation between production of the melanotic mass and cause of death remains obscure.

Four different strains of D. melanogaster have been found which show a high resistance toward insecticides of the organo-phosphorous type. Such resistance has not

which is inhibited to 25-30% by LD-50. As no other enzyme system is known which might cause different reactions to the organo-phosphorous insecticides, these are probably due to a metabolism of these compounds, which can be accomplished only by the resistant strains.

Rasmussen, Inge E. Ovary transplantation in "sex-ratio" stock of D. bifasciata.

In D. bifasciata an abnormal sex ratio showing cytoplasmic inheritance has been described (Magni, 1953). In order to test the possibility of transmitting the "sex-ratio" cytoplasmic particles from SR (sex-ratio) flies to N (normal) flies, ovaries marked with y from N larvae have been transplanted into SR wild larvae according to the scheme:



In 6 females the implanted ovary was functioning. The results are given in the table.

No.	Progeny			
	♀♀ +/y	♂♂ +	♀♀ y/y	♂♂ y
1	-	-	1	-
2	15	-	5	3
3	10	-	5	4
4	.80	-	22	28
5	14	-	3	1
6	106	-	6	3

The offspring of the implanted N ovaries consisted of males and females, whereas the host ovaries produced only females. In order to control the sex-ratio condition of these flies, they were cultured singly. The females descending from the implanted ovary proved to be all N, the females from the host ovary all SR. As a reasonable conclusion, we exclude the possibility of infection of normal D. bifasciata with the sex-ratio cytoplasmic particle by means of ovary transplantation.

Röhrborn, G. Induction of pseudotumors in D. melanogaster by injection of acellular extracts.

On the basis of experiments by Harnly (1954), an attempt has been made to induce melanotic pseudotumors in 120-hour-old larvae of the strains "Berlin wild" and y w, by injecting the following agents: (1) acellular extract of 120-hour-old larvae of the strain tu<sup>8</sup>; (2) an analogous extract of the strain tu<sup>49h</sup>; (3) an analogous extract of the pseudotumor-free strain cl1; (4) an extract like (1) except that the larvae were reared on a dead-bakers'-yeast medium (designated as tu<sup>8x</sup>); (5) Waddington's insect Ringer's solution. Since hereditary and induced pseudotumors

outlast pupal and adult stages as pigmented rest bodies, the adults were dissected, and the presence of observable pseudotumors was recorded. The search for pseudotumors was negative in the "Berlin wild" strain as host. On the contrary, as shown in the table, a considerable number of melanotic pseudotumors could be found in all experiments in which *y w* larvae were used as hosts. The results of experiments 1-3 are homogeneous ( $P = 0.4$ ). The differences between the collective experiments 1-2-3-4 and 4-5 are significant ( $P < 10^{-6}$  and  $P = 0.0025$ ). In another control experiment it was attempted to induce pseudotumors by inserting the empty injection needle only. The outcome was negative. The induced pigmented growths correspond in their morphological characteristics to the hereditary melanotic pseudotumors in *Drosophila*, the so-called "Drosophila tumors." Thus the experiments show that pseudotumors in *Drosophila* can be caused by unspecific agents.

Expt.	Donor	No. adults dissected	% adults tumorous
1	<i>tu<sup>8</sup></i>	72	77.8
2	<i>tu<sup>49h</sup></i>	38	73.7
3	<i>ell</i>	38	65.8
4	<i>tu<sup>8x</sup></i>	89	43.8
5	Ringer's solution	87	18.4

Sakai, Kan-Ichi, Hiraizumi, Y., Narise, T., and Iyama, S.  
Experimental studies on migration in *D. melanogaster*.

By means of a set of four "population tubes" (see Technical Notes), migration of flies was studied with *D. melanogaster*. A certain number of flies of the Samarkand strain were kept for one day in a tube,

and then the tube was connected with three new tubes through three passages. The first series of experiments dealt with the relation between the number of flies that migrated and the length of time during which migration was allowed to occur. These experiments gave the following results:

Initial no. flies in original tube	No. of expts.	% flies migrating to new tubes after (hours)						
		6	24	48	72	96	120	144
100-149	5	1.84	0.95	2.25	3.32	5.52	6.51	7.21
150-199	4	14.66	11.02	13.31	14.51	20.95	22.47	25.01
200-249	4	14.92	14.47	15.41	18.47	23.50	24.59	24.00
250-300	5	19.59	21.35	24.39	27.55	29.90	32.46	32.91

It seems that a good deal of migration occurred within 6 hours, if the number of flies in the original tube was more than 150, although migration continued even after 6 hours.

The second series of experiments explored the problem of whether migration was dependent on the number of flies present in the original tube. In these experiments, number of migrating flies was counted two days after the new tubes were connected. The results of these experiments were as follows:

	Initial number flies in original tube					
	0-50	50-99	100-149	150-199	200-249	250-300
No. experiments	2	2	6	7	6	5
% migrating flies	5.88	0.65	1.89	23.47	26.49	24.93

The results indicate that migration occurred largely as the effect of pressure of population density, and that the critical population size was around 150.

Another experiment appeared to demonstrate that different species of *Drosophila* behaved differently with regard to the population density associated with migration.

Fandler, L. Additional evidence on the role of the centromere in determining disjunctional patterns.

From a cross of females carrying an attached-X chromosome, one arm of which was  $y^+$  In(1)sc<sup>S1</sup>  $y$  In(1)EN and the other  $y^+$  In(1)sc<sup>8</sup>, and no homolog, by males carrying the YSX.YL,  $y$  B chromosome with no homolog, a number of non- $y$ , B males were recovered. The females had been  $\text{H}_2$ -irradiated with about 2000r. Although a stock was established from each of the recovered B males, in all but one case the normal allele of  $y$  segregated independently of the YSX.YL chromosome in males, suggesting that possibly one of the tips of the attached-X (including  $y^+$ ) had capped an autosome; such stocks were discarded. In the one remaining line, on the other hand, the normal allele of  $y$  separated regularly from the YSX.YL chromosome in males, and from an attached-X chromosome in females, suggesting here that  $y^+$  was present on a free centric chromosome fragment (designated FR-IV). The following data on the segregation of FR-IV have been collected: (1) the progeny from a cross of  $y^2$  su-wa w<sup>a</sup> bb/FR-IV X YSX.YL,  $y$  B/O included 317  $y^2$  su-w<sup>a</sup> w<sup>a</sup> bb females, 391 B males, 6 su-w<sup>a</sup> w<sup>a</sup> females, and 14  $y$  B males; (2) from a cross of  $y^2$  su-wa w<sup>a</sup> bb/O X YSX.YL,  $y$  B/FR-IV, 171 su-w<sup>a</sup> w<sup>a</sup> females, 224  $y$  B males, and no exceptions were recovered. This regularity in the separation of FR-IV from the sex chromosomes parallels that of X-chromosome duplications generally. The following additional information about FR-IV has been obtained: (1) FR-IV carries bb<sup>+</sup>; (2) it carries neither the ci nor the ey locus; and (3) translocation tests for linkage between  $y^+$  and either chromosome II or chromosome III were negative.

From the nature of the cross from which FR-IV was recovered, and from the information about its composition, it appears very unlikely that the heterochromatic fragment carries a sex-chromosome centromere (for, indeed, no sex-chromosome centromeres were available unless a minimum of four breaks had been produced by the irradiation), and it seems exceedingly likely that the centromere involved comes from chromosome IV. Crossovers of essentially this type have been reported numerous times in the past. The origin of FR-IV can then simply be explained by supposing a heterochromatic eucentric exchange between chromosome IV and the sc<sup>8</sup> or sc<sup>S1</sup> tip of the attached-X, yielding a chromosome of the composition:  $y^+$  sc<sup>8</sup> (or sc<sup>S1</sup>) bb<sup>+</sup> from the X chromosome, plus the basal region and centromere of chromosome IV. If this is so, then the regular separation of FR-IV from the sex chromosomes would support the idea that the centromere itself is not active in determining the patterns of segregation.

Sandler, I. Segregation in females heterozygous for T(1;4)BS.

Sandler, I. Segregation in females heterozygous for  $T(1;4)BS$ . In order to determine the frequencies with which the various gamete types are formed in females carrying  $T(1;4)BS$  and a normal X chromosome, females of the constitution  $y\ w^3\ m\ f\ car/sc\ w^{e-2}\ cv/BS$  were mated to males carrying  $T(1;4)BS$  with the markers  $y\ sc\ cv\ m/BS\ car/sc^3.Y$ . Progeny resulting from this cross can be readily classified as to their zygotic constitution, and hence the constitution of the various female gametes that are formed can be determined. The evidence indicates that in parental females bearing the translocation and the normal X, gametes arise from alternate, adjacent I and adjacent II segregations. However, whereas in most translocations analyzed to date alternate segregation seems to occur with a frequency somewhat exceeding the sum of the frequencies with which adjacent I and adjacent II segregations occur, with adjacent II segregation occurring less frequently than adjacent I, estimations of the frequencies with which such segregations occur in a  $T(1;4)BS$  heterozygote indicate that adjacent II segregation occurs with about the same frequency as does alternate segregation. Rough determinations of these frequencies are: alternate segregation, 43%; adjacent I, 18%; adjacent II, 38%. The complementary products resulting from each of the segregations are produced with the same frequency.

Shiomi, T. Lethal effect of  
 $C^{21}$  mutant of *D. virilis*.

X-rayed flies (Imaiizumi, unpubl.). It has been found that  $C^{21}$  has a recessive lethal effect at the egg stage. The normal embryonic development of homozygous  $C^{21}$  advances until about 12 hours after egg laying. Before germ-band contraction, lethal eggs show a delay in development. In lethal eggs the germ band never contracts, and gradually ceases to exist. This lethal effect is very interesting in connection with the mechanism of germ-band shortening in the development of *Drosophila* eggs.

Shiono, T., and Kitazume, Y.  
Changes in glycogen content  
during early embryonic develop-  
ment in *D. melanogaster*.

were used; glycogen determinations were made by the anthrone method. Results are given in the table. (U = unfertilized egg; C = stage of contraction after 30 minutes of egg laying; B = blastodermal stage; B N = normal blastema of attached-X strain; B L = Nullo-X embryos at the normal blastodermal stage; G = gastrulation stage.)

The glycogen content in eggs of D. melanogaster, and its changes during early embryonic development, have been worked out. Five hundred dechorionated eggs of Oregon-R-S and of an attached-X strain

mg/500 eggs	Oregon-R-S				Attached-X			
	U	C	B	G	U	C	B	N L
Lyoglycogen	0.268	0.187	0.167	0.152	0.250	0.185	0.185	0.177
%	100.0	69.8	62.3	56.7	100.0	74.0	70.8	74.0
Desmoglycogen	0.017	0.025	0.022	0.020	0.020	0.025	0.017	0.019
%	6.4	9.3	8.1	7.5	8.0	10.0	7.6	6.8

As developmental stages proceed, there is a marked tendency toward decrease of lyoglycogen content, in contrast with desmoglycogen content. In the attached-X strain, Nullo-X embryos cannot develop to the blastodermal stage, and the glycogen content of these lethal embryos at the B stage of normal development remains unchanged from that at the C stage. It is considered that during the early embryonic stages lyoglycogen may be utilized for embryonic development.

\*Steffensen, Dale, Fingerman, Louis, and Anderson, Lulu F.  
Failure to increase the recessive lethal frequency in *Drosophila* with ethylene diamine tetra acetic acid (EDTA).

The metal chelating agent, ethylene diamine tetra acetic acid, (EDTA) was added to food of *D. melanogaster*, presumably to bind calcium. The hypothesis was that mutational events might be induced, since calcium and magnesium deficiencies have been shown to produce

chromosomal aberrations in the plant *Tradescantia paludosa*. Wild-type males of an inbred Oregon-R stock were raised on EDTA food. Adult males were mated to Muller-6 females ( $y^2$  In(1)sc<sup>8</sup> d<sup>1</sup>-49 v w<sup>a</sup> f). Female progeny were tested in creamers for recessive lethals. The table shows the results with five concentrations of EDTA in food (oatmeal, cornmeal, dried yeast, agar, Karo, and molasses) and as a control, food without EDTA. The pH of each food treatment was adjusted to 7.5 with KOH. A chi-square test showed no significant difference between the pooled EDTA treatments and controls.

Females should be grown on EDTA food to determine their frequency of lethals, because of the increased crossing-over response obtained with female larvae grown on EDTA food.

(Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

EDTA molarity in food	Number of lethals	Per cent lethals	No. of male chromosomes
.004	3	0.6	499
.005	0	--	1243
.006	0	--	1099
.007	0	--	646
.008	1	0.2	500
Pooled EDTA treatments	4	0.1	3987**
Control	6	0.3	2171

\*\*Chi-square test between control and pooled EDTA treatments, P. = 0.3-0.2.

Strangio, V. A. Studies on a wing mutant in *D. melanogaster*.

In a stock derived from a single sepia-eyed female trapped in Queensland during 1951, a new character affecting wing morphology appeared during the following year. The new mutant resembles blistered (bs) and balloon (ba) in that the gross structure is deformed by the production of fluid-filled

bubbles during the process of wing expansion on emergence from the pupal case, but gene expression is more extreme than in either published account. Penetrance is complete at the three experimental temperatures--20°, 25°, 30° C. Expressivity varies, ranging from a small blister at the tip of the fifth longitudinal vein to the extreme case in which the whole wing is converted to a fluid-filled sac. Wings with small blisters display a variable degree of plexus formation and extra venation, especially in the submarginal and second posterior cells. A marked temperature sensitivity is apparent: at the higher temperature (30° C) blistering is reduced; whereas at 20° C all wings have warped surfaces, heavy chitinization of the intervein material, and chaotic venation. Semidominance, manifested at 25° C in the female heterozygote alone as small vein-flecks at the posterior crossvein and along the second longitudinal vein, is extended at 20° C to both male and female. A striking interaction of the heterozygote with the third-chromosome dominant Delta (Dl<sup>3</sup>) produces in the majority of cases a single large blister in the center of the wing, presumably due to a threshold effect. Cross-over data placed the mutant locus in the second chromosome, slightly to the left of blistered and balloon; and, pending the results of allelism tests, the term edematous wing (ew) has been adopted. Further tests of fertility, fecundity, relative viability and temperature-effective period in relation to gene action during development are in progress.

Taira, T., and Nawa, S.  
Quantitative measurement of riboflavin, folic acid, and uric acid during metamorphosis of some mutants in D. melanogaster.

been investigated.

The quantity of riboflavin and folic acid during the metamorphosis of v, bw, and se strains was measured by the method of bioassay. The results are shown in the first table below (r/mg; PR = prepupae, PS = postpupae).

	v		bw		se	
	PR	PS	PR	PS	PR	PS
Riboflavin	10.0	10.0	20.0	18.0	70.0	120.0
Folic Acid	.75	.60	.80	.65	.60	.70

As the table shows, no definite change in amount of folic acid during metamorphosis was observed. As regards riboflavin, no definite change in amount during metamorphosis was seen in the v and bw strains. The se strain contains much more riboflavin than the other strains, and its increase from the prepupal stage to the postpupal stage is quite significant.

We also measured spectrophotometrically, using uricase, the micro-amounts of uric acid during metamorphosis of strains Oregon-2, v, se, sed, bw, and w. These are shown in the next table (r/mg).

(See table on following page.)

	3rd-instar Larvae	Prepupae	Postpupae	Young adults
Oregon-2	.47	3.00	3.80	2.30
v	.35	2.30	3.00	1.90
se	.42	2.00	3.00	1.90
sed	.25	3.50	4.70	3.60
bw	.54	3.40	4.70	2.60
w	.27	2.30	3.90	5.30

These results seem to show that the formation of uric acid is independent of the increase in amount of pteridine during metamorphosis. Therefore we seem to be justified in concluding that the amount of none of these substances parallels the production of pteridine.

Flies of the bw stock, grown on a culture medium with an excess of  $N^8$ -azaguanine, which inhibits the metabolism of purines, have a larger amount of isoxanthopterin than do flies cultured on normal medium.

These evidences suggest that the precursor of pteridine is some kind of purine. Further details concerning this problem will be published elsewhere.

Takada, H. Notes on the vertical distribution of *Drosophilidae*.

This survey was made on Mt. Rishiri (altitude, 1719 m.) on a small island located in the northern part of Hokkaido, in August 1956. Three traps baited with fermenting banana and tomato were set up at each altitude. The scheme of the collection and the results are summarized in the table.

Species	Altitude (m.)							
	0- 100	100- 300	300- 500	500- 700	700- 900	900- 1100	1100- 1300	1300- 1719
<i>Parascaptomyza</i>								
<i>disticha</i>	2							
<i>Hirtodrosophila</i> sp.			1			4		
<i>Drosophila coracina</i>	1							
<i>D. testacea</i>	3	5	16	24	47	30	23	16
<i>D. bifasciata</i>	53	59	70	43	15	11	2	
<i>D. nigromaculata</i>	3	2	1		3			
<i>D. transversa</i> (III)	1	1						
<i>D. sp. (quinaria</i> gr.)	1	1						
<i>D. histrio</i>	2	1	2	2				
<i>D. immigrans</i>			1					
<i>D. funebris</i>	4							
<i>D. busckii</i>	2							
<i>D. sp.</i>				1				
<i>D. sp. (obscura</i> gr.)	3	4	2					
Total	75	73	93	70	69	41	25	16

0-1100 m., forest zone; 1100 m-1300 m., shrub zone; 1300 m-, alpine plant zone.

Tattersfield, F. Resistance to insecticides.

shown that (1) resistance to DDT is increased by the addition of yeast or of Casein to the food medium; (2) population pressure on food supplies affects resistance in the adults--high populations cause a decline--but the pattern of resistance differs between the two sexes; (3) high population density in the larval stage affects the weight of the adults, but resistance to DDT is not necessarily correlated with the size of the insect.

Thoday, J. M. A line responding to selection in one direction only.

One of our lines under selection for sternopleural chaeta number has given results showing that a population can be heterozygous for loci that, though readily exploited by selection in one direction, cannot be exploited by selection in the opposite direction. The line is homozygous *dp* and is maintained as four single-pair cultures in each generation. The four cultures are kept as one population by a rotational mating system. The line began with 19.3 chaetae per fly and was selected for high chaeta number. The mean rose steadily to 24.5 chaetae at generation 21, and then rapidly to 28 at generation 24, then more slowly to a plateau of 30 chaetae at generation 30. It is now at generation 50.

The main interest lies in a subline taken out at generation 9 when the mean was 22.6 chaetae. At this point, of course, the main line was still heterozygous for the loci that ultimately raised it to 30 chaetae. The subline was selected for low chaeta number for 15 generations and showed no response to this back selection. It was then (generation 24) selected for high chaeta number, and its subsequent history was almost identical with that of the main line. It rose steadily to 24.3 chaetae at generation 35, rapidly to 28 at generation 42 and more slowly thereafter. It is now at 29 chaetae and may have reached a plateau.

It is clear that the subline, despite the progress made in the first 9 generations, was, during the period of back-selection, heterozygous for loci that could be exploited to raise chaeta number but could not be exploited to lower it. The observation is relevant to interpretations of genetic homeostasis.

Thompson, Peter E. A bilateral mosaic gynandromorph with second and third chromosomes marked.

the second and third chromosomes contributed by the female parent. The left side of this individual was Curly, non-Plum, non-dumpy, Stubble, and non-Ultrabithorax, and lacked sex combs. The right side was Plum, dumpy, non-Curly, Ultrabithorax, and non-Stubble, and had sex combs. The left side of the abdomen was distended, but the genitalia appeared to be entirely male. Mating behavior was apparently male, although actual copulation with females was not observed. This individual is believed to have arisen by double fertilization of a binucleate egg.

From the cross of an *Ins(2)SM1*, *al Cy sp<sup>2</sup>* / *Ins(2)Pm*, *dp b Ph ds<sup>53k</sup>*; *Ubx<sup>130</sup> e<sup>S/C</sup> Sb* female with a *dp/dp* male, an offspring was obtained which was gynandromorphic and a bilateral mosaic for markers on each of

Tinderholt, Victor E., and Hinton, Taylor. The ability of heterochromatin to produce position effects when removed to a new location in the chromosome.

In(2LR)40d had a region of heterochromatin from the base of 2R placed in the 27 region of 2L. This arrangement produced a position effect upon the eye. From this arrangement, In(2LR)IICQ was derived. It has the heterochromatin in question located between regions 23A and 23B and

lacks the eye effect. The question was asked, does this heterochromatin exert an effect on the wild-type alleles of any of the genes in region 23. To test this, as many recessives as possible, suspected of being in the area, were collected and crossed to In(2LR)IICQ. To date, positive results have been obtained in one case. The gene "rubroad" (rub), whose phenotypic expression is broadened and shortened wings and which is at 5.0<sup>+</sup> on the linkage map, consistently shows a broadened and shortened wing when heterozygous with In(2LR)CQ. The expression is less than that of the homozygous recessive mutant. In all cases, series of wings were permanently mounted on microscope slides and measurements made. The genotypes rub/+ and +/In(2LR)CQ give a wild-type wing. This shows that heterochromatin removed to a new location in the chromosome can still act to produce V-type position effects. It also locates the gene rub on the salivary chromosome as being to the right of and near 23B.

Toyofuku, Y. Chromosomal polymorphisms found in natural populations in Hokkaido.

An investigation was made of polymorphisms of the salivary-gland chromosomes in natural populations of Hokkaido. Eleven different kinds of chromosomal variations

represented by heterozygous inversions were found in nine strains of the following six species: D. bifasciata, D. immigrans, D. melanogaster, D. nigromaculata, D. sordidula, and D. virilis. Six of these types were found in D. immigrans collected in the University Botanical Garden at Sapporo; in all the specimens the aberrations occurred in one or two chromosomes.

Ulrich, Hans. Effect of oxygen on the mutagenic action of X-rays on uncleaved Drosophila eggs.

Drosophila eggs of wild-type females mated to Muller-5 males were X-rayed at the age of 10-20 minutes, in air or in nitrogen, with the same dose of about 1500 r (50 kv, 10 ma; FD, 50 cm; time of exposure, 192 sec.). Each surviving F<sub>1</sub> female adult was mated to a single F<sub>1</sub> male derived from an untreated F<sub>1</sub> egg. Each F<sub>2</sub> offspring should consist of +/+ females, M5/+ females, + males, and M-5 males. Absence of + males or M-5 males indicates the induction of a sex-linked recessive lethal in the normal X or the Muller-5 X of the irradiated F<sub>1</sub> egg from which the F<sub>1</sub> mother in question developed. The results summarized below demonstrate that absence of oxygen during irradiation reduces the mutagenic action of X-rays.

Treatment	No. of eggs	Hatching %	Surviving adults %	No. of F <sub>2</sub> cultures with more than 20 adults	No. of F <sub>2</sub> without (or with single) adults + males	No. of F <sub>2</sub> without (or with single) adults M-5 males	Sex-linked recessive lethals %	
							No. of F <sub>2</sub> without (or with single) adults	No. of F <sub>2</sub> M-5 males
<u>X-rayed</u>								
in air	8611	5.4	2.8	112	5	8	5.8	
<u>X-rayed</u>								
in N <sub>2</sub>	3495	21.7	14.4	221	9	8	3.85	
Control, untreated	1200	93.5	87.0	100	-	-	0.0	

Van Alten, Pierson J. The induction of sex-linked recessive lethals by high-energy electrons.

The following data are of interest in connection with the induction of sex-linked lethals by high-energy electrons. The source of electrons was a General Electric million-volt resonant-transformer type of electron-beam machine, with an energy range of 0-1 Mev., with a root mean square of 0.707 Mev. Adult (Oregon-R inbred) males, 4 days old, were irradiated and immediately mated to Muller-5 ("Basc") virgin females; and after 96 hours these parent flies were removed from the bottles. The results suggest that the rate of mutations to dose was not linear as with X-ray-induced mutations. At higher doses (3000, 4000, and 5000 rep.) the high-energy electrons seem to be less effective than X-rays.

Dose in rep.	No. treated males	No. X chromosomes tested	No. lethals	Per cent mutation
0	40	858	6	0.7
1000	30	608	26	4.3
2000	8	129	7	5.4
3000	31	341	26	7.6
4000	44	555	36	10.1
5000	20	48	8	16.6

(I wish to thank Dr. A. S. Fox for his suggestions and help with this study, and Mr. D. E. Wiant and Mr. R. Nicholas of the Department of Agricultural Engineering for operating the electron beam machine.)

Wakahama, K. A new type belonging to the genus *Amiota*.

Thirty flies belonging to this type were collected at Numanohata in Hokkaido, by means of banana traps, in June 1954. The presence of milky white areas at the wing base and humerus is characteristic of the subgenus *Amiota*. External characteristics closely resemble *A. gigantia* and *A. leucostoma*; but there are clear differences in the shape of the clasper and the genital arch. A brief description of the male genitalia follows: Genitalia, very large. Genital arch, broad and roundish below. Anterior margin sinuated a little; posterior margin stair-like; heel, right angle. About 26 bristles on lower and middle portions; upper portion has about 9 bristles. Anterior margin and lower posterior margins chitinized. Anal plate separated from arch and very small, with dense bristles. Clasper, one; primary teeth, about 10 arranged in a straight row, with the first one or two shorter. There are about 5 thin bristles above the teeth, also a number of thinner hairs at the outer lower corner.

Welshons, W. J. Dosage experiments with split mutants in the presence of an enhancer of split.

Dr. M. M. Green was kind enough to send the author an enhancer of the sex-linked recessive split. The enhancer is associated with *T(2;3)Xa*. It was utilized in the performance of some dosage experiments,

which are reported here in a preliminary form. When the enhancer is present in females heterozygous for split, the flies have a phenotype quite similar to *spl/spl*. Hemizygous split males have a very extreme split phenotype when the enhancer is present; homozygous split females show the same extreme expression of split in the presence of the enhancer. These findings were conveyed to the author by M. M. Green in a personal communication. Since one split allele and

wild allele of split yielded a split phenotype in the presence of the enhancer, it was of interest to see if the effect could be eliminated by the addition of another wild-type allele. Therefore, homozygous  $Dp(1;1)Co$  females were crossed with  $y^2$   $su-w^a$   $w^a$   $spl$ ;  $Xa-En$ -spl males. The  $F_1$  females,  $Dp(1;1)Co/y^2$   $su-w^a$   $w^a$   $spl$ ;  $Xa-En$ -spl, had one mutant allele and two wild alleles of split in the presence of the enhancer. They had a split phenotype, which was similar to that of homozygous split females.

The  $Dp(1;1)Co/y^2$   $su-w^a$   $w^a$   $spl$ ;  $Xa-En$ -spl females were then crossed to  $y$   $w^{def}$   $rst^3$ ;  $Dp(1;2R)w^{5lb7}$  males (Lefevre, DIS-26). The  $y^2$   $su-w^a$   $w^a$   $spl$ /  $y$   $w^{def}$   $rst^3$ ;  $Xa-En$ -spl females with one wild and one mutant allele were compared with  $y^2$   $su-w^a$   $w^a$   $spl/y$   $w^{def}$   $rst^3$ ;  $Xa-En$ -spl/ $Dp(1;2R)w^{5lb7}$  females which had two wild-type alleles and one mutant allele. It could be seen by this comparison that the females with two wild alleles were less extreme in phenotype than those with only one wild allele, although in both cases the expression of the mutant phenotype was within the range of expression found in  $spl/spl$  females. Because the addition of an extra wild allele does not greatly alter the expression of the phenotype when the enhancer is present, the effect was not noticed in the initial cross.

As might be expected from the above cross,  $y^2$   $su-w^a$   $w^a$   $spl$ ;  $Xa-En$ -spl/  $Dp(1-2R)w^{5lb7}$  males with one wild and one mutant allele are less extreme in phenotype than  $y^2$   $su-w^a$   $w^a$   $spl$ ;  $Xa-En$ -spl males which have no extra wild allele present.

In previous experiments with different  $N/spl$  heterozygotes it had been observed by the author that the pseudodominant expression of  $spl$  is somewhat reduced as compared with that of  $spl/spl$  homozygotes. This is not the case with  $N/fa$  heterozygotes, in which the expression of facet is more extreme than in homozygous facet flies. In the one case tested so far,  $N^{Co}/spl$ ;  $Xa-En$ -spl, as expected, was less extreme than  $spl/spl$ ;  $Xa-En$ -spl.

These results can be understood if the mutant allele split is a mutant by virtue of the fact that it causes to be produced some substance which is different than that substance produced by the wild allele, and if the wild-type and split alleles are in competition for the same substrate. Then, if the production of a split substance is enhanced, the production of a + substance is indirectly inhibited. Conversely, an enhanced production of the + substance caused by the addition of wild alleles would inhibit the production of a split substance.

Widmer, Elmer Andreas. A rediscovery of the original rp mutation in *D. melanogaster* Oregon-R-C stock. (Master's thesis)

Widmer, Elmer Andreas. A rediscovery of the original rp mutation in *D. melanogaster* Oregon-R-C stock. (Master's thesis)

A study was made of the rotated genitalia in Oregon-R-C males. Investigations involved fertility, effects of temperature, mode of inheritance, location of the gene responsible for it in its proper linkage group, its locus on the chromosome, and its relationship to the original rp mutation described in 1929.

Matings between rotated-genitalia males and Oregon-R females proved that only males with a degree of rotation between  $10^\circ$  and  $60^\circ$  are fertile. The appearance of the mutant is correlated with temperature; it has a much reduced penetrance at low temperatures, and its penetrance increases progressively with an increase of temperature from  $18^\circ$  C to  $26^\circ$  C. Flies with a  $90^\circ$  rotation were the most numerous in cultures kept at  $26^\circ$  C. The degree of

rotation was determined by means of an ocular protractor.

The gene for this condition is carried on the third chromosome at approximately locus 95.7. Because of reduced penetrance this position must be considered as only approximate. Tests with the original rp mutation (1929) suggest that the two mutant genes are the same. They agree in being recessive and on the third chromosome, but differ in their position on the chromosome.

#### TECHNICAL NOTES

Annan, Murvel E. A method for collecting eggs from individual Drosophila females.

Individual Drosophila females were placed with two males in 3/4-ounce creamers, which were closed with regular cardboard caps. Large paper straws (1/4 inch in diameter) were cut into lengths of approximately three inches. A length of straw was first dipped in water and then forced into a dish of standard corn-meal-agar-molasses food medium of sufficient depth so that when the straw was withdrawn it would be filled with food for at least an inch. The end of the straw containing the food was then sliced at an angle. Varying the angle of slice would vary the amount of food surface exposed. The knife or scalpel used must be sharp. It was found that the slice was most readily executed when the straw was held on end--food end down. The other end of the straw was flattened and folded to effect a closure. Identification data were written on the folded end of each straw and on the creamer cap with a copying pencil. The exposed food surface was lightly seeded with yeast and then inserted through a 1/4-inch hole punched in the cardboard creamer cap. A standard paper punch was of the proper size. The caps were secured by application of two or three spots of Duco cement. The brood chambers were stored in an upright position (for economy of space) in 6 x 10 inch baking pans. The pans were placed in an incubator at 25° C, in which a high humidity was maintained to prevent drying and shrinkage of the food. The straws could be changed as often as the investigator wished. This method was effective in minimizing the escape of flies while changing food straws.

Bennett, Jack. Inexpensive population cages.

In this laboratory (Department of Genetics, University of Wisconsin) we have been successfully using population cages made from 1 1/2-pint polyethylene refrigerator boxes (as Montgomery Ward, cat. no. 86C4578D, 12 for \$2.89). These boxes have 4-inch square tops, are 4 inches high with tapering sides, and feature a tight-fitting interlocking cover. Four holes are punched in each of two opposite sides, and one for ventilation in the top. The holes are a very tight fit for our 25 x 95 mm shell vials, which with food are used to fill all the side holes; the top hole is plugged with cotton for ventilation and can be used for a collecting vial to extract samples. Extra cotton-plugged ventilation holes may be necessary with some species, to keep down internal humidity. Food vials are changed one at a time

at five-day intervals for D. robusta, D. virilis, and D. melanogaster, and all three seem to do well. Obviously, more holes could be added or larger boxes used if a cage with greater capacity were needed.

Frosseau, G. E., Jr. Fast green as a useful counterstain for neuroblast and salivary smears.

Zeilinga (1956) has reported the use of fast green as a counterstain for aceto-orcein squash preparations in plant material. The green counterstain improves contrast and inhibits fading of the orcein. His procedure was successful on *Drosophila* smears. In salivaries the nucleolus stains a clear, pale green; but inconsistent results were obtained with the nucleoli in neuroblast preparations. The green background and improved contrast do facilitate screening of brain preparations for division figures. The following procedure was successful. The dissected salivary glands or neuroblasts are fixed in 3:1 acetic acid-alcohol and then placed in 2% aceto-orcein in 70% HAc. After staining is complete, the excess stain is removed and the tissue gently blotted with Kleenex to remove any remaining stain. The tissue is then placed on a clean slide in a drop of 0.1% fast green in 45% HAc and allowed to remain for about 1 minute. Then the material is squashed in the fast green by the usual method. The preparations may be sealed with wax or made permanent by any of the common techniques.

Burton, L., and Friedman, F.  
A technique for the tissue culture of *Drosophila* tumors.

This communication describes the preparation of a nonsynthetic culture medium and its successful application in the culturing of *Drosophila* tumors.

All materials utilized in the preparation of the tissue cultures are autoclaved for 30 minutes. The tissue culture medium is prepared as follows. A sample (5 grams) of fresh 96-hour larvae (a genetically tumor-free strain) is ground in a mortar and suspended in 15 ml of Ringer's solution. This mixture is centrifuged at 35,000 x g for 30 minutes at 0° C in a Spinco refrigerated centrifuge. The resultant supernatant is then filtered through a Swinny filter.

All culture procedures are conducted in an enclosed hood, which has been washed with 70% alcohol and bathed in ultraviolet light (6 hours). The larvae containing pigmented tumors are washed in four consecutive baths of Ringer's salt solution, three baths of 70% alcohol, and a final bath of sterile Ringer's solution. The tumors are carefully removed without injury to the larval gut (a tear in the larval gut could liberate yeast cells and bacteria which might contaminate the culture). The liberated tumors are drawn into a constricted glass micropipette. The tumor is lodged at the constriction in the pipette, and is washed by drawing sterile Ringer's solution through the pipette. The tumor is placed, with a drop of sterile Ringer's solution, upon a cover slip. This fluid is withdrawn and a drop of the culture medium added carefully (to prevent the tumor from floating). The cover-slip is inverted over a depression slide, and the culture is sealed with liquid paraffin. The hanging-drop culture is incubated at 25° C.

After one week of incubation, the tissue culture can be opened, the old culture medium removed, and fresh medium added. Through the utilization of these techniques, *Drosophila* tumors in culture, with and without normal tissue, can grow.

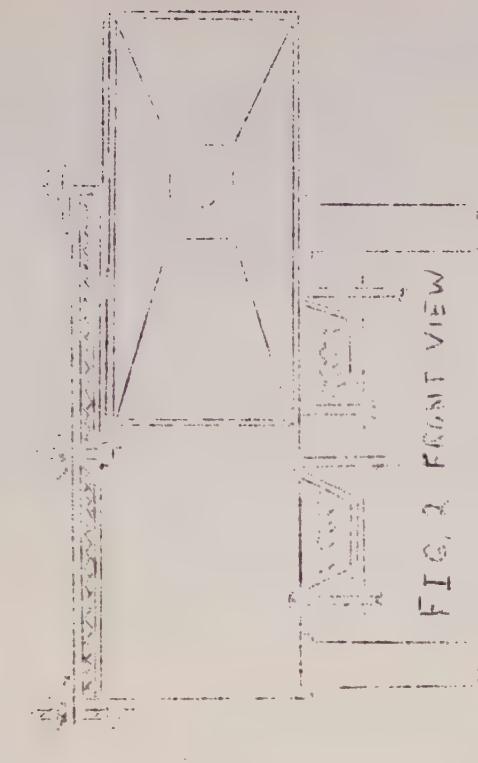


FIG. 2 FRONT VIEW

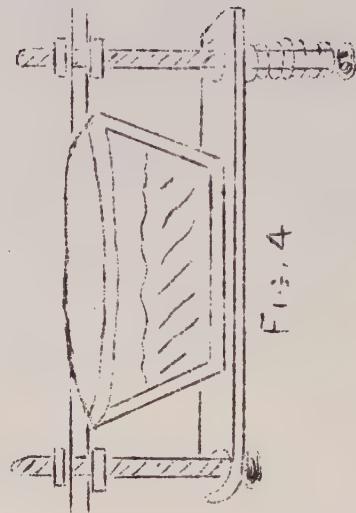


FIG. 4

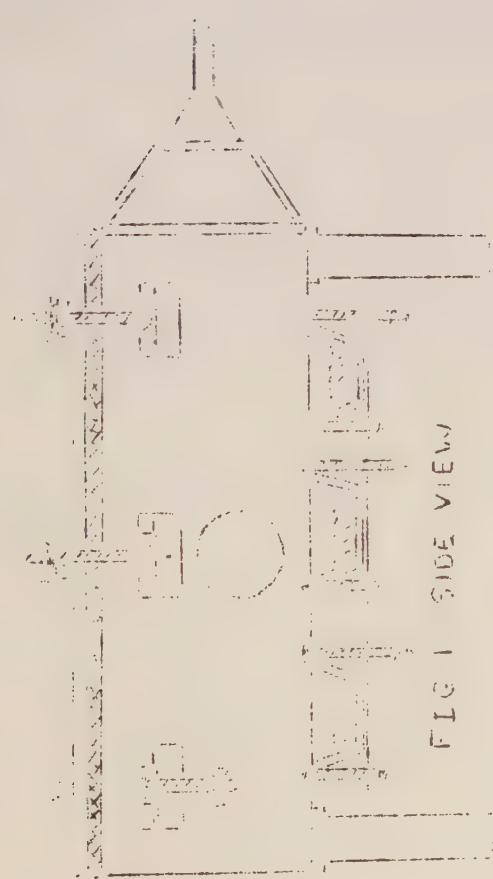


FIG. 1 SIDE VIEW

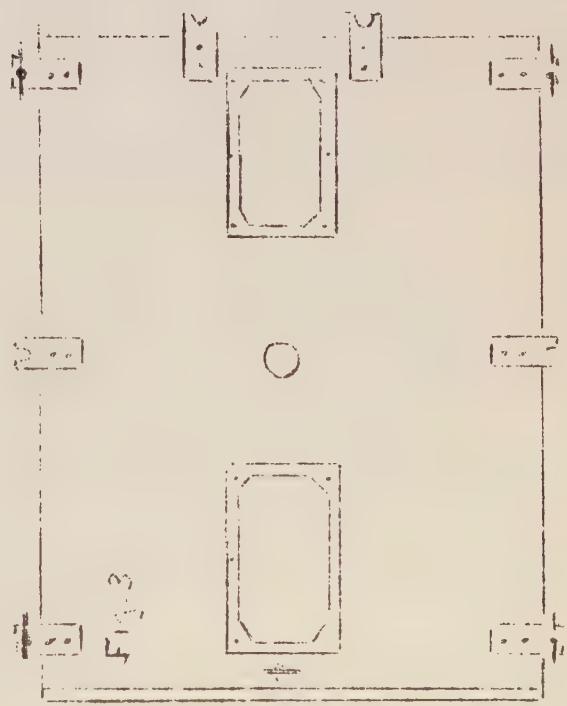


FIG. 3



Friedman, Frank, Ostrander, Frank, Burton, Lawrence, and Solomon, Stanley. A technique and collection cage for the acquisition of large numbers of specific-age larvae.

The acquisition of large numbers of larvae of a specific age is vital to certain experimental procedures. To meet this need, a collection cage was built to provide a continual supply of specific-age larvae. The method of collection is as follows. The cage is sealed, and stenders of media are fitted into place (figs. 1 & 4). A funnel is inserted into the hole in the top of the cage (fig. 3, top view of upper plate) and adult flies are passed through. After the flies have been placed in the cage, the funnel is removed and the hole stoppered. After a given egg-laying period, the stenders are removed and replaced. The egg-laden stenders are placed in a 25° C incubator for the desired developmental time. Flies may be removed from the cage, without etherization, and collected. This is accomplished by replacing the blank panel by a funnel panel (fig. 2) and forcing the flies through the funnel. The cage may then be dismantled, cleaned, and immediately readied for use.

The cage is constructed of 1/4-inch plexiglas. The L-shaped brass brackets are notched to accommodate a 10-32 Fillister head screw, with wing-top nuts. These brackets are fitted atop the upper plate (fig. 3). The cut-outs atop and in the sides of the cage are fitted with 60-mesh wire screen by means of aluminum panels (figs. 1 & 3). A gasket (rubber weather stripping, 5/16" x 3/8") is fitted to the upper edges of the cage, so that a tight seal is insured when the upper plate is fixed to the cage. Holes (3 1/2") are cut into the bottom plate and counterbored from the under side to accommodate stenders (62 mm diameter). These stenders are held in place, and rest upon an aluminum strip (fig. 4). The pressure of the compression spring upon the metal strip keeps the stender firmly seated (fig. 4). Stenders are removed by swinging the metal strip outward, and new stenders are seated into place by hooking the metal strip onto the supporting screw (fig. 4).

Dimensions and other details for the construction of this collection cage will be furnished upon request.

[The accompanying mimeographed illustrations were kindly supplied by the authors.]

Kuroda, Y. Synthetic medium for the tissue culture of *Drosophila*.

Although a brief description of this technique for tissue culture was given in DIS-28 (p. 127), some improvements have been made in the synthetic medium to obtain better growth of tissues, and detailed directions for preparing the synthetic medium are given below. The ingredients were dissolved separately to make the following ten stock solutions, which were stored at 0° C.

1. Stock solution A: NaCl, 14.0 gm; KCl, 0.4 gm;  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 0.04 gm;  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ , 0.2 gm;  $\text{NaHCO}_3$ , 0.1 gm;  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ , 0.4 gm; glucose, 1.6 gm; water to 100 ml.

2. Stock solution B: Casein hydrolyzate, 10.0 gm; tryptophan, 0.2 gm; cystine, 0.2 gm. These substances were dissolved by gently heating in 100 ml of 0.075 N HCl.

3. Stock solution C: Cysteine, 52 mg; water to 10 ml.

4. Stock solution D: Thiamine, 1.0 mg; riboflavin, 1.0 mg; pyridoxine HCl, 2.5 mg; niacinamide, 2.5 mg; Ca-pantothenate, 1.0 mg; water to 1000 ml.
5. Stock solution E: Ascorbic acid, 50 mg; water to 10 ml.
6. Stock solution F: Choline HCl, 50 mg; inosite, 5 mg; p-aminobenzoic acid, 5 mg; water to 1000 ml.
7. Stock solution G: One-hundredth ampoule of vitamin A palmitate (30 mg in 1 ml water); water to 3000 ml.
8. Stock solution H: One ampoule of vitamin B<sub>12</sub> (10 µg in 1 ml water) was used as it stood.
9. Stock solution I: Sodium acetate, 10 mg; glutathione, 2 mg; glutamic acid, 20 mg; water to 10 ml.
10. Stock solution J: DPN, 2 mg; water to 100 ml.

The culture medium was prepared by mixing together equal proportions from the above stock solutions, just before using. After the mixture had adjusted to pH 7.2, 0.5 mg/ml of PNA (from yeast) was added. This culture medium was sterilized by filtration through a Seitz filter.

Lewontin, R. C. A simple technique for measuring egg laying.

carbon black is added to the hot mixture to make it quite black. The medium is spread on the surface of ice-cream sticks, which can be purchased in lots of 10,000 for about \$8.00. This is an extremely inexpensive, disposable carrier for the medium. Spreading the food on sticks is best done by a plastic mustard dispenser of the flexible polyester type. The aperture in the nozzle should be slit so that a slight pressure on the container will disperse a ribbon of cornstarch medium of the right width onto the stick. Several hundred sticks can be prepared in a short time, and if kept in a moist container in the refrigerator they will keep for several days. The blackened cornstarch provides a smooth flat surface, making visibility of the eggs good without refocusing of the microscope. The linear arrangement of the medium on the stick reduces errors in counting, as the sticks may be moved from left to right in a continuous motion without having to retrace steps.

Nawa, S., and Taira, T.  
Simple microdetermination of uric acid by using uricase fermentation.

minutes at 100° C. After cooling, the solution is centrifuged at 4000 rpm for 2 minutes. Ether is added to the supernatant, and centrifuged. As a measurement solution, 4.5 ml of 4 mg crystalline uricase dissolved in 100 ml of glycine buffer is used. A sample of 0.5 ml of the test solution is measured spectrophotometrically at wave length 292 m $\mu$ , and incubated at 37° C. After 4 hours, the solution is again measured spectrophotometrically. Thus, the amount of uric acid is calculated from the value of standard fermentation of uric acid by using the same uricase solution.

The egg-laying surface is made from boiling cornstarch and water. Use the recipe for thick cornstarch which is printed on the back of the box. Enough

Fifty individuals of D. melanogaster at various stages are used as material. After the material is homogenized in a solution of 5 ml glycine buffer (pH 9.4), it is boiled in a water bath for 5

Oftedal, Per. Handling radioactive flies.

When measuring radioactivity in flies it is usual to put the fly or flies in a small gelatin capsule, which is then placed under the counter. In some experiments it has been found desirable to make several measurements per day, and in this case the many etherizations necessary in order to place the flies in the capsules and remove them becomes the limiting factor to the study of normal physiology.

To avoid these etherizations, the following two little gadgets have been designed (figs. 1 & 2). Figure 1 shows the apparatus for putting flies into the capsule. It utilizes the same principle as was recently recommended by King and Wilson (J. Exptl. Zool. 130, 1955). The procedure is as follows. One half of the capsule is placed in position I. The vial is next inverted over the funnel, and the fly shaken down into the capsule. The knob of the stopcock (A) is turned, thus confining the fly to the lower compartment. The other half of the capsule is inserted (II), the stockcock is opened, and the upper half of the capsule is pushed home. After it is ascertained that the capsule is really closed, it is pushed out with a piece of blunt 1.5-mm wire and is ready for the counter.

To remove the fly from the capsule to the vial, the apparatus in figure 2 is used. The apparatus is put on top of the vial, the capsule is inserted in the hole across the top (B), and the entrance is stoppered (C). With two needles, one straight and one hooked, the two halves of the capsule are pulled apart through the slit in the top, and the fly is shaken down into the vial.

The two gadgets are best made from Perspex, which can be polished so that one can see the flies at all stages of operation. Special care should be taken while drilling the holes, to avoid blistering. The apparatus should be made as light as possible for ease in handling, but at the same time the walls should not be thinner than 5-7 mm, to give protection against soft beta emitters, and even a certain amount against hard beta.

The apparatus may be of help even in handling nonradioactive flies which should not be etherized. With slight modifications--e.g., one or more extra holes (F) in the stopcock--it should enable one to separate pairs of flies, etc., as well.

Thanks are due to our health physicist, Dr. Per Grande, for helpful discussion.

(See figures 1 and 2 on following page.)

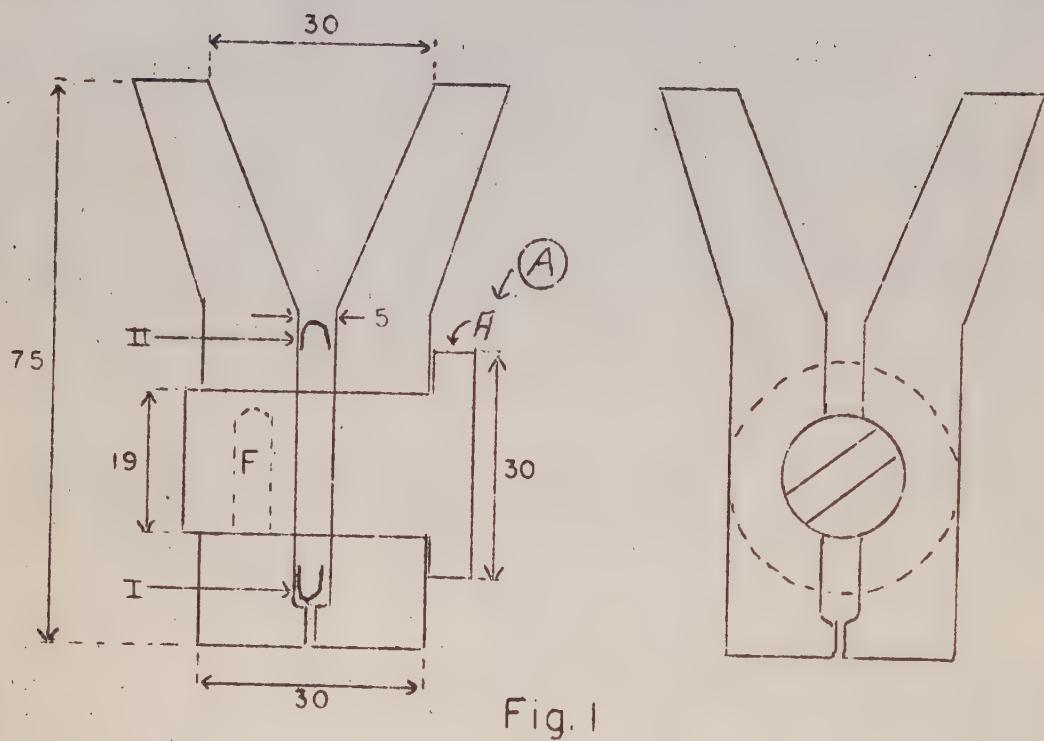


Fig. 1

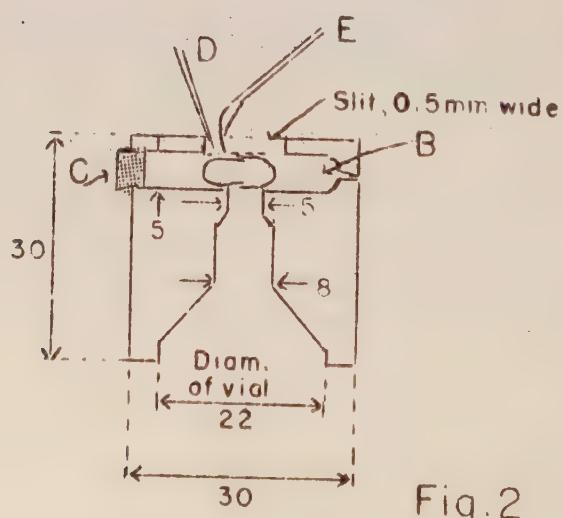


Fig. 2

Oftedal, Per. Measuring the volume of injection needles.

It is somewhat difficult to calculate the volume of the glass injection needles used in *Drosophila* work on the basis of measurements under the microscope, because the internal diameter of the needle is hard to determine. One can measure the diameter of drops of liquid expelled into another liquid, the two being immiscible. But here, again, one may find measurement difficult, since the drops are rather larger than is suitable for that method. This note has the purpose of bringing to the attention of *Drosophila* workers the possibility of using tracer methods for volume measurements. Even if the counting equipment is not available in most genetics institutes, the necessary apparatus will be found in almost any chemistry or physics laboratory. The procedure is simple and involves very little work.

The volume in question is measured out from a very weak solution of some radioactive isotope, preferably a fairly high-energy beta emitter, e.g.,  $P^{32}$ . It is expelled into a drop of water on one of the usual plaquettes used in most scalar assemblies. Thereafter the solution is diluted 1:100 or 1:1000, and a known volume--e.g., 5  $\mu$ l--of this solution is pipetted onto a similar plaquette. After evaporation to dryness, the plaquettes are placed under the G.-M. tube for assay of radioactivity. A comparison of the two activities, corrected for the relevant dilution factor, gives the volume with a high degree of exactitude.

To minimize inaccuracies due to adsorption to the pipette walls, the solution used should contain inactive carrier isotope. If one tries to expel from the pipette onto the dry plaquette, one often finds that the drop creeps up along the outside of the pipette instead of settling on the plaquette, thus yielding inaccurate measurements.

Paik, Y. K. An improved technique, using the phase microscope, for studies of male genitalia in *Drosophila*.

Since Salles (1948) reported an effective technique for making preparations of male genitalia, it has been widely used among the workers in this field. However, as examination of some details is unsatisfactory and cumbersome in preparations made by Salles's technique, it was modified by Malogo (1952), who reported a coloration method for better examination. His preparations were made by Salles's technique and were stained with safranin of Johansen. This method was found to be effective in showing better contrast between the minor structures of the genitalia, but it was apt to bring about distortion or some damage to the genitalia. An improved technique which we have found is based on some modification of Salles's preparation technique and the use of the phase microscope. It is described below.

(1) Preparation technique: (a) Separate the terminalia in phenol. (b) Boil in an adequate amount of 10% sodium hydroxide on a slide glass. (c) Clarify in phenol. (d) Let the genitalia stand in creosote for more than one hour. (e) Mount in Diaphan. (f) Examine the preparations under the phase microscope.

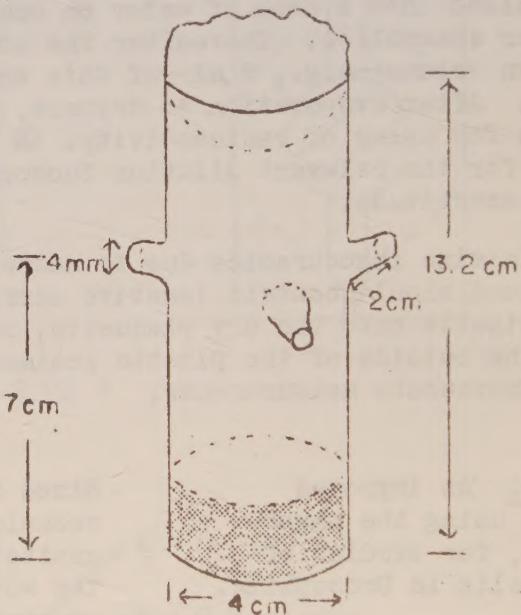
(2) Observation: For observation of the preparations, we used a phase-contrast microscope (Olympus Optical Co.). Among the objectives, we found that Dark (A-) contrast-Low (PL 10x in our microscope) gave the best results for examining and drawing the genital structures. For illumination we used a 6 v, 5 A transformer and a ground blue filter. Use of the phase microscope with the above-described preparation technique gave us excellent results.

This technique is much better than any other, with or without staining, in terms of showing clear-cut contrast between structures, and of avoiding distortion or damage to the genitalia. We found it completely satisfactory for purposes of examining or drawing the genital structures.

Sakai, Kan-Ichi. "Population-tubes": an apparatus for the study of population genetics in *Drosophila*.

This is an apparatus devised for the study of population genetics in *Drosophila* and allied insects. It consists of a variable number of unit tubes, each 4 cm in diameter and 13.2 cm in height.

The tube has a bottom, and contains an appropriate amount of food. The other end is stuffed with cotton. The characteristic feature of this tube is its three radial branches, 4 mm in diameter, protruding from the side.



Connecting these unit tubes with each other by short small tubes of vinyl resin, we can investigate changes in an insect population due to migration, competition, and so forth.

Travaglini, E. C. A method for collecting and counting large numbers of *Drosophila* eggs.

The following method, devised at the suggestion of Dr. Jack Schultz, makes it possible to obtain approximately 2000 eggs from each collection box at three-hour intervals during a two-week period.

Two hundred and fifty to three hundred females with approximately twice that number of males are placed in cotton-stoppered, quart Mason jars equipped with stainless steel trays, 5 x 2 x 3/8 inches in size. These trays contain portions of a molasses-agar medium made from 420 ml molasses, 35 ml Moldex, 2800 ml water, and 70 gm Bacto-agar, upon which two lumps of yeast approximately 1 cm in diameter are spaced. In this way, the females are fed adequately for as much as a two-week collection period, and continue to lay well for most of this time.

For collection, the bottom of the jar is gently tapped on a rubber pad so that the flies fall to the bottom; the tray upon which the eggs were laid is removed and a fresh one inserted in one operation. Since very little egg laying occurs the first half-hour after a new tray is placed in the jar, two hours is taken as a minimum period when large numbers of eggs are desired. The eggs are brushed off the molasses-agar with a soft camel's-hair brush into a Petri dish containing 70% alcohol. By gently swirling the dish, the eggs are concentrated at the center of the dish and the adherent medium washed away. This process is repeated with fresh 70% alcohol until all the contaminants are removed. Then the eggs are transferred to a 3:1 alcohol-ether solution contained in a vial, which is immersed in a dry ice-acetone bath. After the eggs are frozen, they are stored at -40° C until used.

For counting the eggs, the following method was devised with the help of Dr. Jerome J. Freed. Before being transferred to a vial for freezing, the eggs are dispersed in a single layer just covered by 70% alcohol over the bottom of the Petri dish. They are then photographed against a dark background at 1x magnification. From this negative superposed on a similar negative taken from a millimeter grid, 3x enlarged prints are made. Meanwhile, the eggs are swirled again in 70% alcohol, transferred to a 3:1 (v/v) alcohol-ether solution, and put into a calibrated Bauer-Schenck centrifuge tube whose tip from 0 to .05 ml is filled with paraffin to form a cushion for the eggs. The eggs are centrifuged ten minutes at 2000 rpm and their volume is measured. Finally, they are transferred to a vial and stored as described above.

From the photograph, the number of eggs can be counted. By using the counts and the volumes of eight different egg collections, it was found that one *D. melanogaster* (Oregon-R) egg has a volume of  $28.5 \pm .4 \times 10^{-6}$  ml. Thus, for subsequent egg collections, the number of eggs collected could be determined by measuring only their volume. This entire procedure, from the time of removing the egg tray from the jar to the time of freezing the egg, takes twenty minutes or less.

## TEACHING NOTES

Burdick, A. B. Duplicate sex-linked factors with about 28% recombination.

We have a stock homozygous for both *rb* and *g*(A-42). Since *rb* and *g* are almost indistinguishable and *rb g* has about the same phenotype (which we call "dull"),

these genes serve as duplicate factors for the "dull" phenotype. Genes *rb* and *g* are linked on the sex chromosome, with about 37 map units between them. I ask my advanced class to make the cross: dull(*rb g*) ♀ x + ♂, from which they obtain (one student's result) 382 + ♀♀ and 316 dull ♂♂. I ask them to mass-mate the *F*<sub>1</sub> to obtain: 304 dull ♀♀, 168 + ♀♀; 275 dull ♂♂, 156 + ♂♂, or about 2 dulls : 1 wild-type in *F*<sub>2</sub>. In addition, I ask them to make the original cross reciprocally and, again, to mass-mate the *F*<sub>1</sub> to obtain: 0 dull ♀♀, 399 + ♀♀; 256 dull ♂♂, 127 + ♂♂. In interpreting these data they soon see that lethals and viability factors will not explain the high frequency of dull types in *F*<sub>2</sub> and the obvious sex-linkage. The only reasonable explanation that fits these data is duplicate factors, sex-linked with, in this case, about 28% recombination.

## MATERIALS REQUESTED OR AVAILABLE

Haruo Kurokawa, of the Department of Biology, Tokyo Metropolitan University would like to receive reprints of papers on speciation in *Drosophila*.

Mutant ♂. (See "Melanogaster - New Mutants") Byron R. Kadel, 77-A Fenway North, Baltimore 21, Maryland, will be happy to supply stock of *cn vg dg* to anyone interested.

I. H. Herskowitz (Jordon Hall, Indiana University, Bloomington, Ind.) is preparing Bibliography on the genetics of Drosophila. Part III. It is planned to include the literature from 1951 through 1956 and any titles not included in the two previous Parts, and to have a title index. He would appreciate receiving references to any abstracts, papers, or theses not included in Part I or II or in the current or previous issues of DIS.